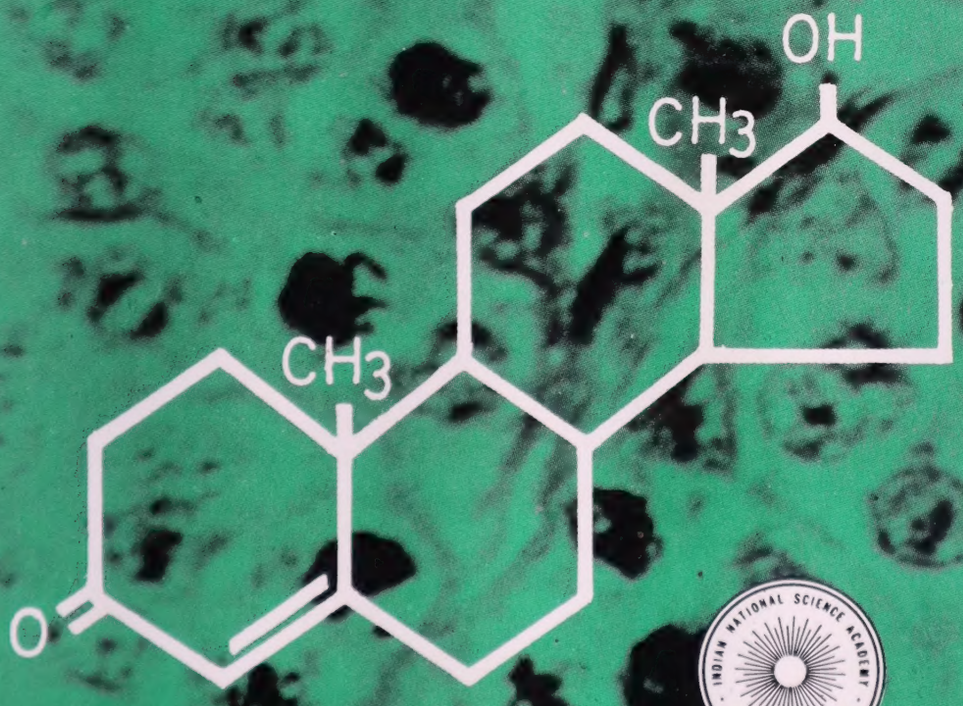
ANATOMY, HISTOLOGY AND BIOCHEMISTRY OF MAMMALIAN TESTIS AND EPIDIDYMIS

L S RAMASWAMI



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A REVIEW

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CONTENTS

1. INTRODUCTION

The female reproductive system has been studied in great detail from a long time for providing the necessary clues towards fertility-control. Now the pendulum has swung towards the male side. The testis and its duct system are the targets for solving fertility-control in the male. It is necessary, therefore, that students of reproductive biology have a good knowledge of this gland. With this in view, I have tried to give a fairly comprehensive account of the male gland and its duct system culling information from recently published papers and not going back to the dawn of 'testicology' (Steinberger & Steinberger 1972). The use of antiandrogens as useful drugs in controlling male fertility, is also discussed.

2 DEVELOPMENT OF THE TESTIS

2a *The Classical Concept*

The primordial gonocytes (some 30–50) arise from the yolksac endoderm and migrate by ameboid movement to the genital ridges which appear along the mesial edges of the mesonephros. Probably, a chaemotactic inductor of the genital ridge attracts them as described in chick embryos by Dubois (1966). Alternatively, the inductor may be steroidal in nature (Baillie *et al.* 1966), but the ridge has no set up for steroidal synthesis. All the same it shows the enzyme hydroxysteroid dehydrogenase connected with steroid synthesis. The region where these gonocytes concentrate becomes the gonad. Cells from the nearby *coelomic* epithelium invade the mesenchymal area and form the gonadal blastema. The medullary sex cords arise from this blastema and they show both gonocytes and somatic elements. The rete cords also arise from the blastema. Below the surface epithelium, a thin layer of connective tissue is formed and this is the future tunica albuginea. The surface epithelium with gonocytes disappears and thus a contingency of the cortex forming ovary-like structure is obviated. Which one of the bipotential gonadal primordium or *anlage*—the cortex or the medulla should gain predominance is governed by the genetic set up of the embryo. Both contain primordial genital cells.

The numerous experiments conducted by Neumann and his co-workers (Neumann *et al.* 1970) have led them to state that during early development of vertebrate embryo, there is always in the embryo a tendency towards female differentiation whatever the genetic sex may be, supporting Wiesner's hypothesis (1934, 1935) of the so-called 'basic femaleness'. No hormonal influences are needed for development of female sex. On the other hand, the male needs androgen and at least one other factor. The male gonad is identifiable in the human in the 7th week of pregnancy and in the pig, it is at the age of 26 days.

2b *Recent Studies*

The origin of the primary germinal cells is still disputed. Some reports state that they arise from the endodermal cells while others say that they arise elsewhere (mesoderm) and enter the endoderm. According to the ultrastructural studies of Clark and Eddy (1975), in the mouse they do not arise in the endoderm.

The differentiation of the primordial gonad into a male gland is heralded by the formation of the sex cords of pre-Sertoli cells which surround the germ cells (Jost *et al.* 1974). A basal lamina or membrane is noticed surrounding the sex cords. The pre-Sertoli cells are mesenchymatous in origin and very likely not coelomic. The cell cord or sex cord formation starts at the centre where mesonephric tubules are located and it is likely that the rete testis derived from the mesonephric tubules and the tubules themselves are putting forth a diffusible substance which induces meiosis in fetal testis (or ovary), the so-called meiosis-inducing substance (MIS) (Byskov 1978). The male gonad may also secrete a meiosis-preventing substance (MPS) which can arrest female germ cells at meiotic prophase (Byskov & Saxén 1976); it is not yet proven if fetal Sertoli cells are the site of origin of MPS. Wartenberg (1978) classified the early Sertoli cells into 'dark' (arising from central gonadal blastema) and 'light' (coelomic epithelial origin) groups—the former secreting MIS and the latter MPS. It remains to be proven if all these are as stated (see also subsection 7a).

3 ANATOMY OF THE MAMMALIAN TESTIS

It is common knowledge that the location of the testes* is not uniformly the same in vertebrates. There are a number of them with permanently abdominal testes (Fishes, Amphibia, Reptilia, Aves and among mammals, the so-called testicond mammals like the marsupial mole *Notoryctes, Procavia, Heterohyrax,* Cetacea, Proboscidea, the elephant shrews *Elephantulus rufescens, Pterodromus tetradactylus,* Rhinoceros, the bat *Rhinopoma*) and others where the male gonads are scrotal having descended from the abdomen. The vasculature also shows a variation depending upon the species examined. In the vertebrates examined with abdominal testes (inclusive of Amphibia, Reptilia and Aves), there is no pampiniform plexus or vascular cone or complicated internal testicular vasculature (*vide infra*).

3a *Vascularization of the Testis*

A unique feature of the testicular vasculature is that the flow of blood in the testicular artery during its intratesticular passage is pulseless and is also stagnant. This lends itself to cadmium insult on the capillary endothelial permeability. Even the proximal part of the initial segment of the epididymis undergoes the same change (Mason *et al.* 1964). Waites and Setchell (1969) noted that capillaries of body and tail of epididymis are unaffected while those of the head region show 'only transient, possibly indirect changes and do refer to the work of Mason *et al.* (1964).

In the mammals with scrotal testes, the arrangement of arterial and venous blood vessels to and from the testis is unique and may also show variations. The

*The Latin term 'TESTIS' connoted 'witness' or 'spectator' and the word 'testament' is derived from it.

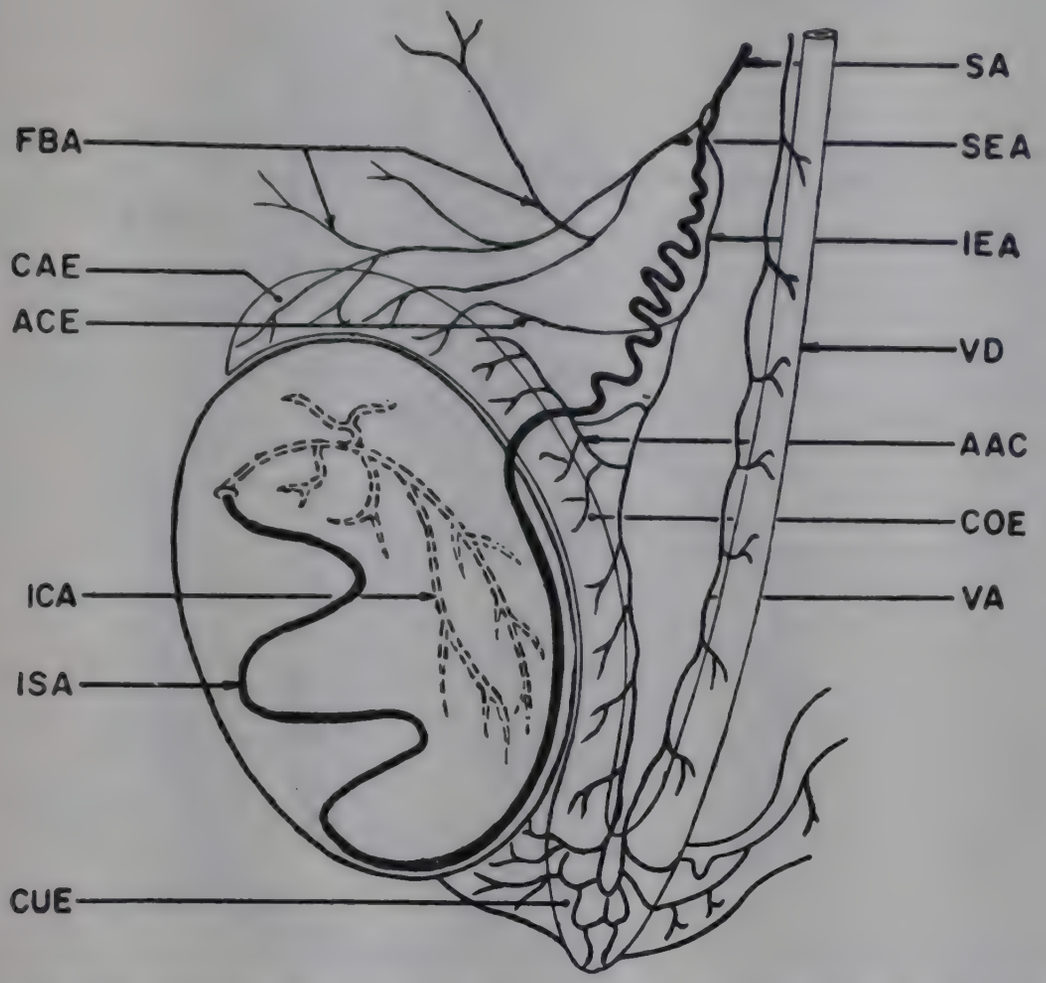


Figure 1. Arterial supply to the Testis and Epididymis of rat (after Oettle & Harrison 1952 *J. Path. & Bact.* **44** 273, modified)

internal spermatic artery (or testicular artery) shows convolutions as it leaves the inguinal canal and the vasculature from here to the testis is disposed in the form of a cone; in man, the extracapsular spermatic artery is relatively straight in the case of Europeans; and coiled in the Africans and Japanese (Hundeiker 1972. Way up in the vascular cone, branches from the spermatic artery are given off to the head of the epididymis (and to the fat body) (figure 1) and these vessels are also convoluted. The inferior epididymal artery supplies the body and tail of the epididymis and unites with the vasal or deferential artery (Harrison 1949); Setchell (1970) records that the deferential artery arises from the hypogastric artery.

The passage of the internal spermatic artery in the testis after passing by the side of the epididymis, shows variation—four patterns are recognized as seen in carnivora ruminants, rat and rabbit. In the rat (figure 1), the artery runs on the deeper surface of the testis without branching and then enters the parenchyma where it branches. The characteristic microvascular pattern in rat develops at the age of 15–30 days (Kormano 1967); intertubular arterioles develop first and these give rise to intertubular capillary network; limited peritubular capillaries are also found. Formation of the centripetal and cetrifugal arteries in the bull testis was described by Hundeiker (1961). The arterial branches run in the testicular septulae which divide the

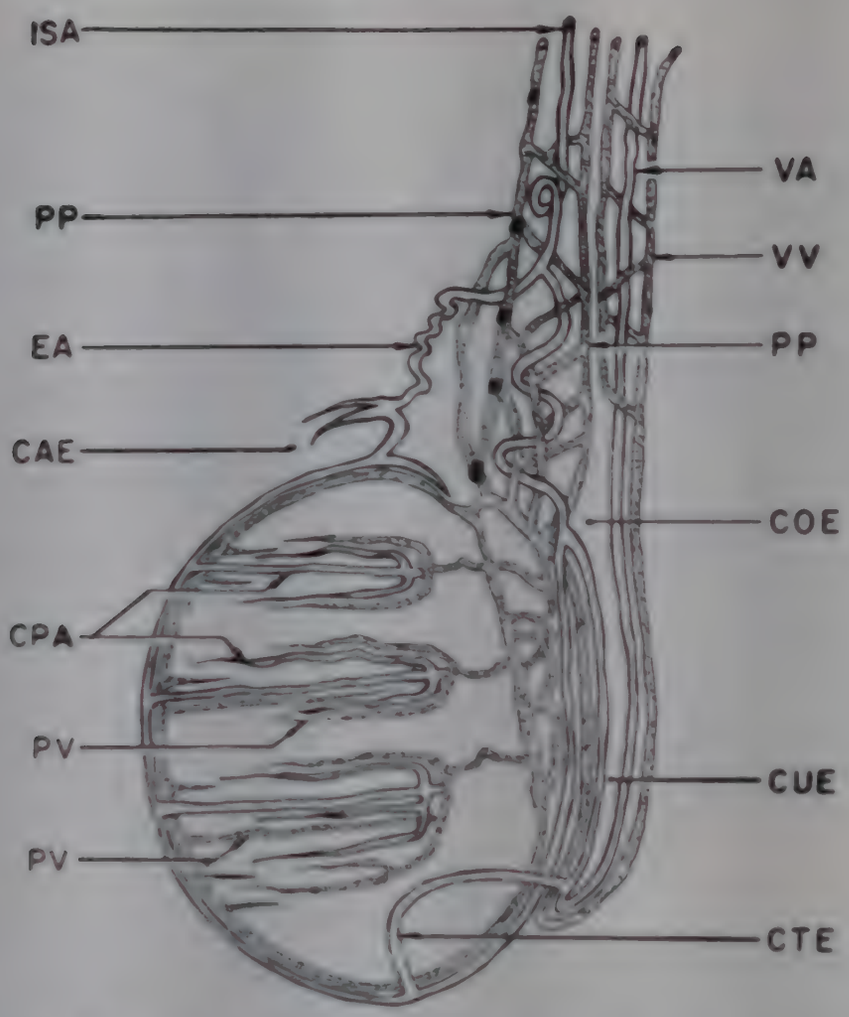


Figure 2. Diagram of the arterial and venous vessels of the testis and epididymis of man; the position of the three parts of the epididymis is indicated by lettering (after Hundeiker 1971, modified)

testes into compartments or lobules. The lymph vessels also reach the mediastinum from the tunica in these septae. Hundeiker(1971) observed a prominent connecting vessel (figure 2, CTE) between the internal spermatic artery and the caudal epididymal artery; this connexion appears to be lacking in larger mammals (Free 1977). In the rat and rabbit, these septulae are poorly developed (Hundeiker 1961) and the lymph capillaries are also absent (Setchell 1970, (figure 3). The veins from under the tunica albuginea as also the central vein from the mediastinum proceed towards the cranial pole of the testis and drain into the pampiniform plexus. Fawcett (1976) described that the pampiniform plexus constitutes a countercurrent system where the arterial blood can lose heat to the cooler venous return so much so the blood entering the testis is pre-cooled. This explanation becomes questionable; for, in the rat the spermatic artery and the plexus are not intimately related and in the monkey they are separated by a thick layer of connective tissue (Harrison & Weiner 1949). All the same, countercurrent exchange of testosterone and other substances can occur between the convoluted veins and the arteries in the pampiniform plexus of many mammals (Free 1977).

In man, the right pampiniform plexus enters the inferior vena cava directly *via* the internal spermatic vein; the left one opens into the left renal vein (Bloom &

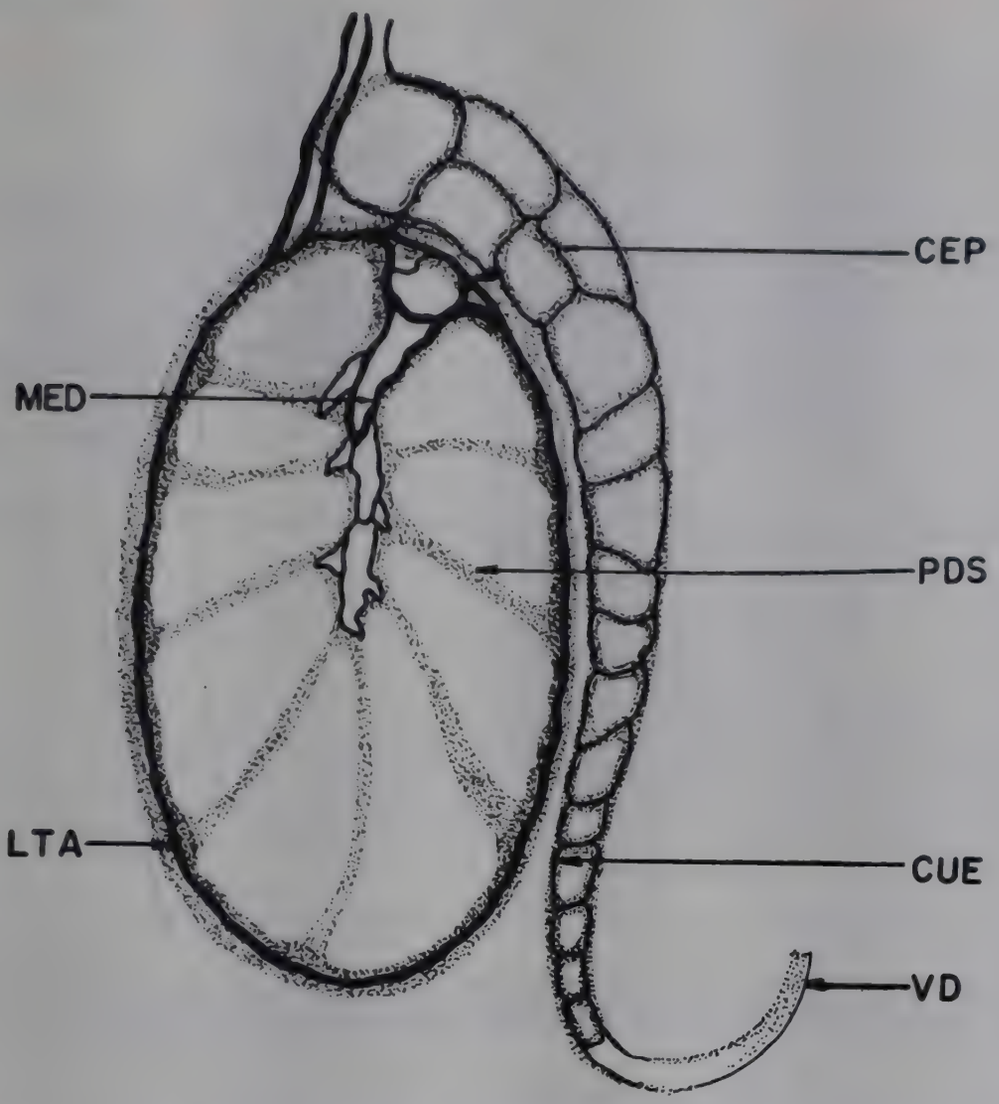


Figure 3. Diagrammatic sectional view of the testis and epididymis of rabbit to show the absence of lymphatic vessels in the poorly developed septulae and their presence in the tunica and epididymis (after Regaud 1897 *C. R. Soc. Biol.* (Paris) **14** 695)

Fawcett 1976). The left pampiniform plexus is more distended than the right one, and the left internal spermatic vein or cremasteric vein may show a swelling bilateral sometimes called the 'varicocoel' above the testis; and this may also result in infertility.

The blood circulation of the two testes is generally considered to be independent of each other; sometimes connexions do occur principally of the cremasteric veins (Brown *et al.* 1967).

3b *The Mediastinum and Rete Testis*

The mediastinum is the vertical incomplete septum projecting from the tunica albuginea to the middle of the testis. Histologically, it is similar to the tunica and supports the rete which is a channel into which both ends of the tubule open; the intratesticular part being formed by them and the extratesticular one by the 'rete blastema' which latter may be of mesonephric origin (figure 4).

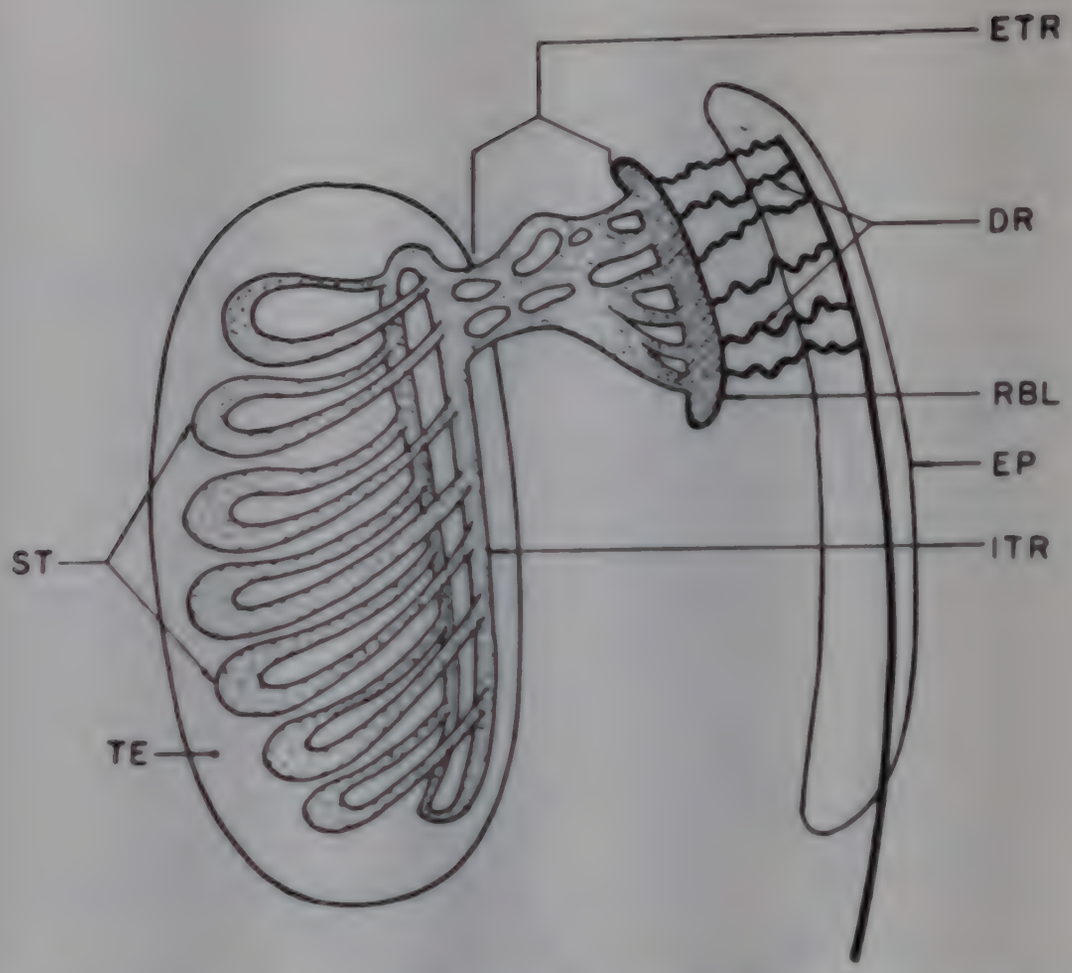


Figure 4. Diagrammatic representation of tubules (sex cords), internal and external rete and the opening of the latter into the efferent ducts (after Roosen Runge 1961, modified)

The disposition of the rete testis varies : it may be central (dog, bull, rabbit, monkey) or peripheral (man) (Arcy 1965), extending along most of the epididymal margin of the testis surrounded by mediastinum connective tissue. The monkey *Innus cyanomolgia* has a central mediastinum according to Messing (quoted by Setchell 1970). In the bonnet monkey as in the rhesus, it is centrally disposed (unpublished data). In the rat, the mediastinum, is lateral (or peripheral, Dym 1980), flat and close to the epididymal head juxtaposed to the tunica (figures 5A & 5B); this pattern is also seen in the mouse (Setchell 1978). The rete from the mediastinum pass through the tunica and enter the ductulus or vasa efferentia and the latter open into the epididymal duct. In man, the vasa efferentia (12 or more) emerge from the testis and after forming 5–13 vascular cones are held by connective tissue and form the head of the epididymis. I have not seen the formation of vascular cones in the vasa efferentia of the hanuman langur monkey whose dissections I have examined. Setchell (1978) described that the normal number of efferent ducts is 3–7, while Prakash *et al.* (1979) described 12–17 ducts arising in the rhesus out of the cranial pole of the testis. In the hanuman langur, 9 efferent ducts are noticed.

According to Setchell (1978), the columnar epithelia of both efferent ducts and rete testis show 'tight junctions' at the luminal border; there is no reference to this

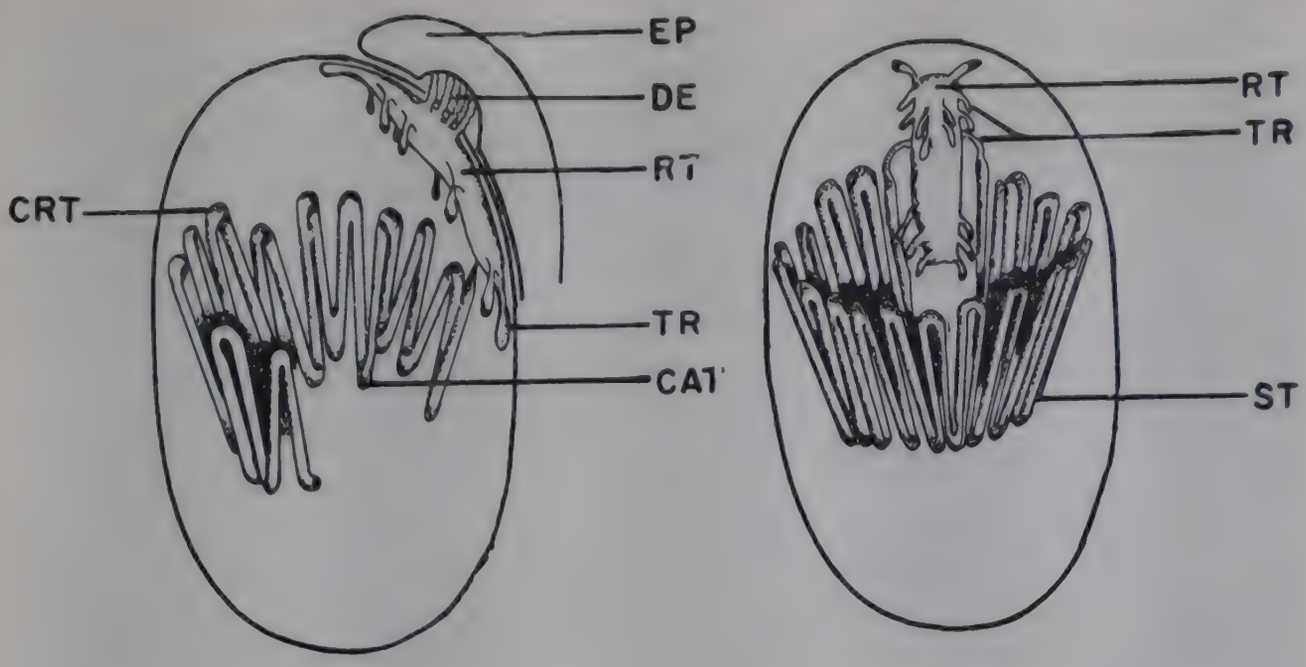


Figure 5 (A) (B) Diagrammatic reconstruction in rat of the diposition of (external and internal) tubules in the form of a series of hairpin bends and the lateral rete testis, efferent ducts and caput epididymis. Each end of the tubule opens into the rete testis by a tubulus; one complete tubule is reconstructed (after Clermont & Huckins 1961, modified)

feature in the descriptions of Prakash *et al.* (1979), possibly because their study was not ultra-structural.

The rete testis cells show microvilli and cytoplasmic vesicles suggesting their involvement in fluid transport.

A transverse section of the testis of a juvenile hanuman langur monkey (8.5 kg) (figures 6a–6d) shows that the tubular lumen is not yet manifest and spermatogenesis has not yet been initiated. The axial mediastinum located more towards the side of the epididymis shows prominently the rete testis consisting of many channels (compare figure 8.2; Setchell 1978). The tall columnar epithelium could also be made out but the tight junctions referred to above are not visible in the light microscope study. In many areas the opening of the tubulus rectus into rete could be seen and the tall columnar epithelial cells here resemble those of the rete.

3c *The Rete Testis Fluid (RTF)*

The flow of the RTF appears to be continuous and there are no circadian variations. It shows very few sperm when compared to ejaculated semen [spermatocrit (i.e., the volume of the cells as a percentage of the fluid volume) 1% approximately]. RTF (when compared with testicular lymph) shows more of K^+ and Cl^- while less of Na^+, Mg^{2+} and Ca^{++}; it has high concentration of ABP (but not so in the boar and monkey) and contains inhibin—a peptide hormone which controls FSH-secretion by the pituitary. Testosterone (T) and 5 α-DHT are also present in the RTF. T is noticed in ram, bull and monkey RTF but very much less than in testicular lymph or blood plasma. Comparatively, the tubular fluid differs from the latter in having more K^+ than RTF. Koskimies (1973) described that the RTF

contained considerable amounts of albumin and globulins in contrast to the tubule fluid. All the same, the rete fluid appears to come from the tubule (Setchell 1978).

3d *The Fetal Testis of the Langur Monkey (Presbytis) and the Testis of the Adult Rat*

The transverse section of fetal testis of *Presbytis entellus* (figures 7a–b) shows that the mediastinum is not exactly central but is more towards the epididymal side. The rete testis appears to proceed towards the tunica albuginea and then into the epididymis (caput or initial lobe) in a passage-like structure which appears to cleave the testis into two parts here. This is not noticed on the opposite side of the testis. The fetal testis is composed of about 24 C-shaped sex cords [a term used by Clermont and Huckins (1961) in the rat testis development but not by van Wagenen and Simpson (1965) in their description of early development of human and rhesus monkey testis and both ends of this C open into rete testis by tubulus rectus. Clermont and Huckins (1961) showed a number of convolutions by reconstruction in which the cranial portion of the hairpin-bend was nearer to the tunica while the other end was located more internally (figures 5a–b). Each tubule looked like a funnel, the narrower portion fitting into the broader part of the next one. No connexions between the convolutions of the tubules were noticed.

4 HISTOLOGY OF THE MAMMALIAN TESTIS

The outermost layer of the testicular capsule which keeps the parenchyma under pressure and makes the blood vessels flattened [figure 8, the scrotal sac (SC) histology is also included], is a thin mesothelial layer—the tunica vaginalis visceral. The cells of this layer* are very thin, non-proliferative and show microvesicles and microvilli. Between the mesothelial cells, there are no desmosomes. Adjacent to this is the most prominent fibrous layer with collagen fibres enclosing fibroblasts. Smooth muscle cells and a few striped muscle fibres are also present (Leeson & Cookson 1974). Blood vessels reaching the testis appear to pierce the tunica albuginea and on its inner face divide and enter the parenchymal tissue, the tunica itself having very few vessels. The tunica extends into the parenchyma in the form of septulae to meet the mediastinum in a majority of mammals. The tunica vasculosa forms an internal investment of testicular parenchyma in all species and may occasionally show a network of blood vessels.

5 THE WALL OF THE SEMINIFEROUS TUBULE

5a *Histology*

The wall is composed of 4 layers (figure 9). Starting from the Sertoli cells and going towards the Leydig cells, one meets with the basement membrane first. Next is a noncellular layer containing glycoproteins, hyaluronic acid and collagen fibres; in the ram this layer may show a lamellar structure. Then there is the inner cellular layer of smooth muscle cells or myoid layer; there is again an external noncellular layer similar to the inner one. Finally, there is a cellular layer of fibroblasts (Fabbrini *et al.* 1977). External to this, there is a loose connective tissue

*It may be difficult to make out this layer under the light microscope

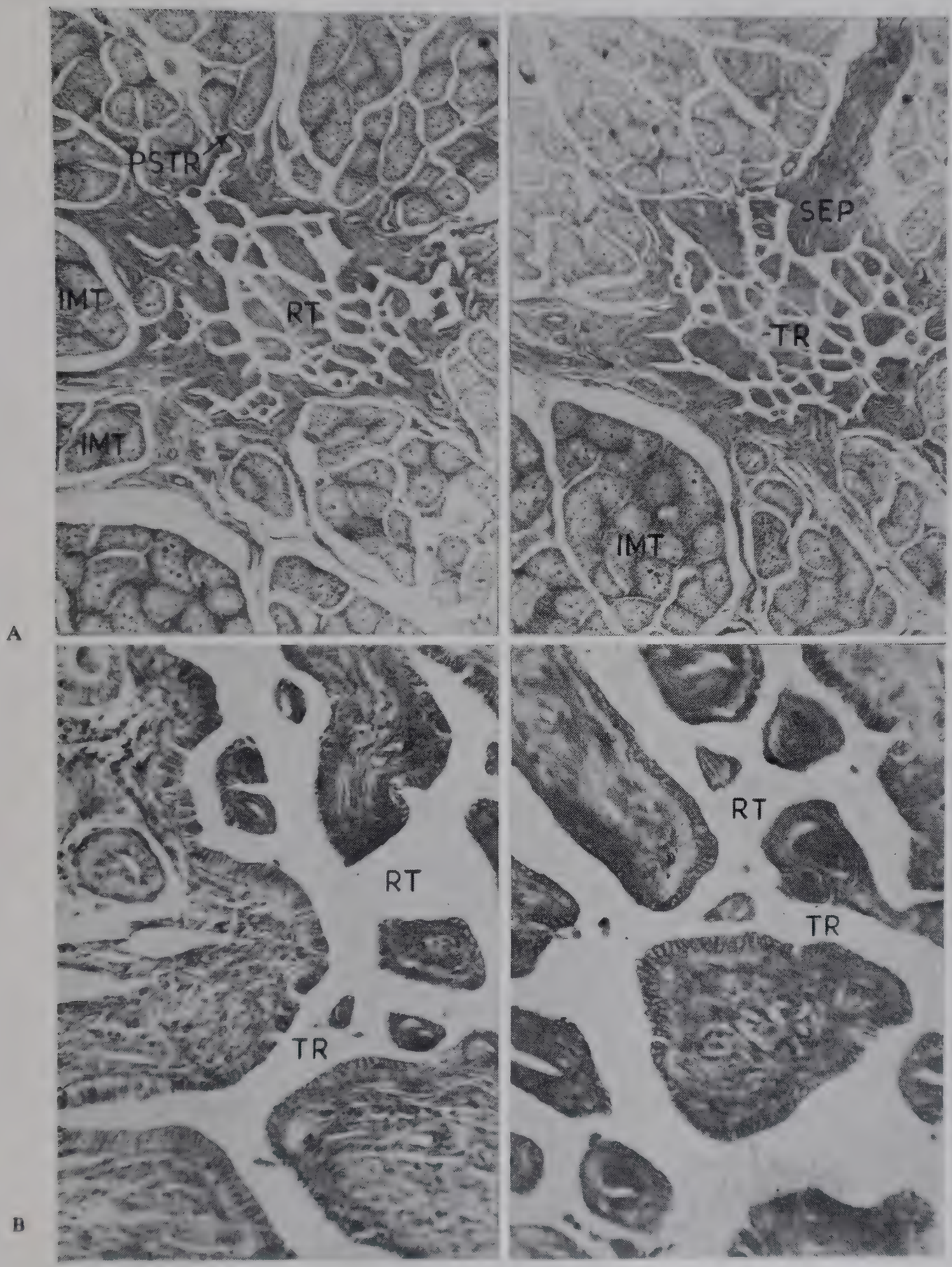
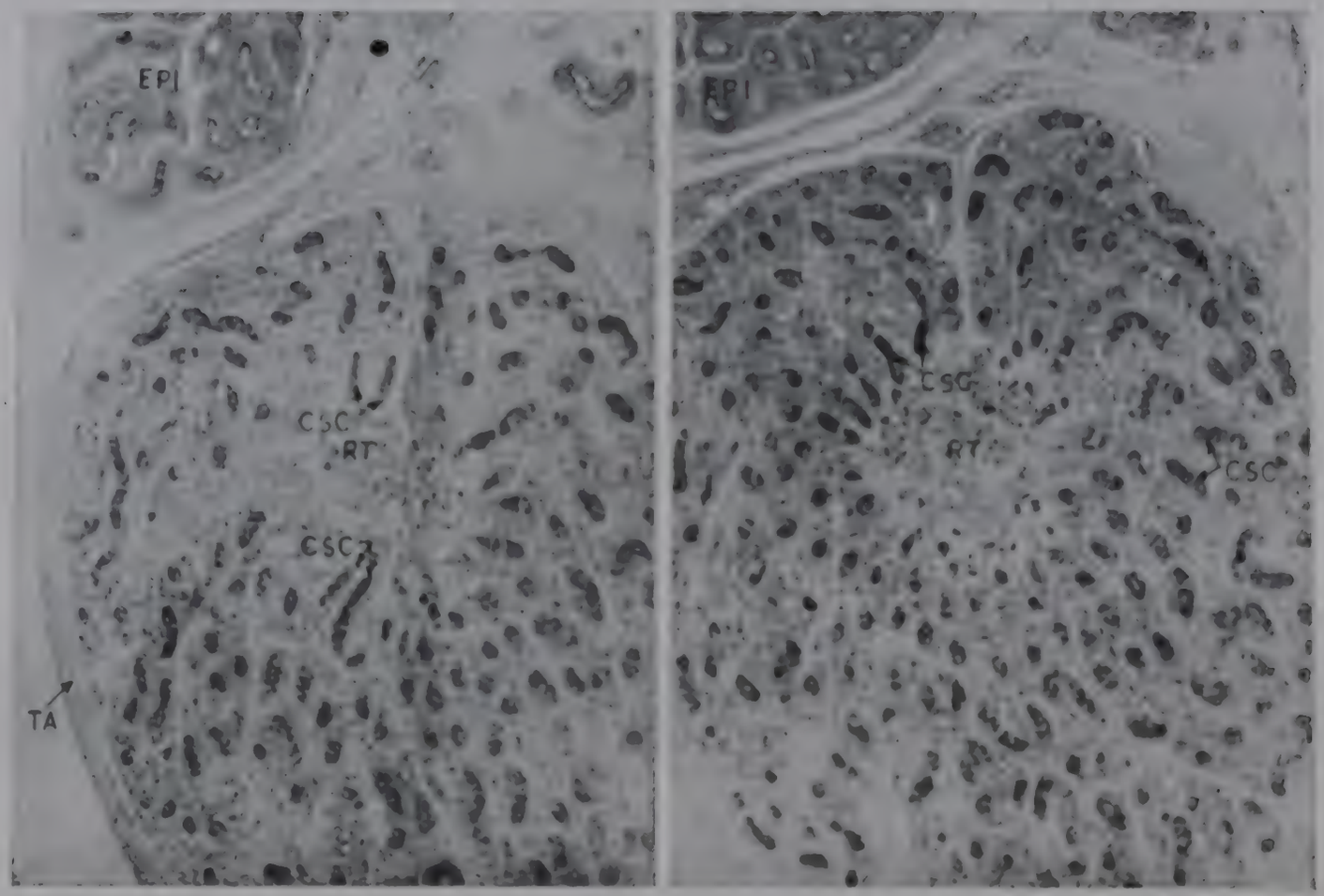


Figure 6.—(A,B,C,D) (Plate 1) Transverse section of the juvenile testis of the langur monkey (*Presbytis entellus entellus*) to show the rete testis and mediastinum. The tubules are still solid. In 6A, the tubulus in continuation with the solid tubule (arrow) is noticed two enter the rete testis; in 6B, a connective tissue septula (thick arrow) is seen running between the tunica and the rete. In 6C and 6D, the tall columnar epithelium of the tubulus and rete can be seen original, × 300, HE



A B

Figure 7. (A) (B) (Plate 2). Transverse section of fetal testis and epididymis of the langur monkey (*Presbytis entellus entellus*) (C.R. : 180 mm) to show the limbs of C-shaped sex-cords, the epididymis and the axial mediastinum, which is more towards the epididymis, original, × 300, H-E

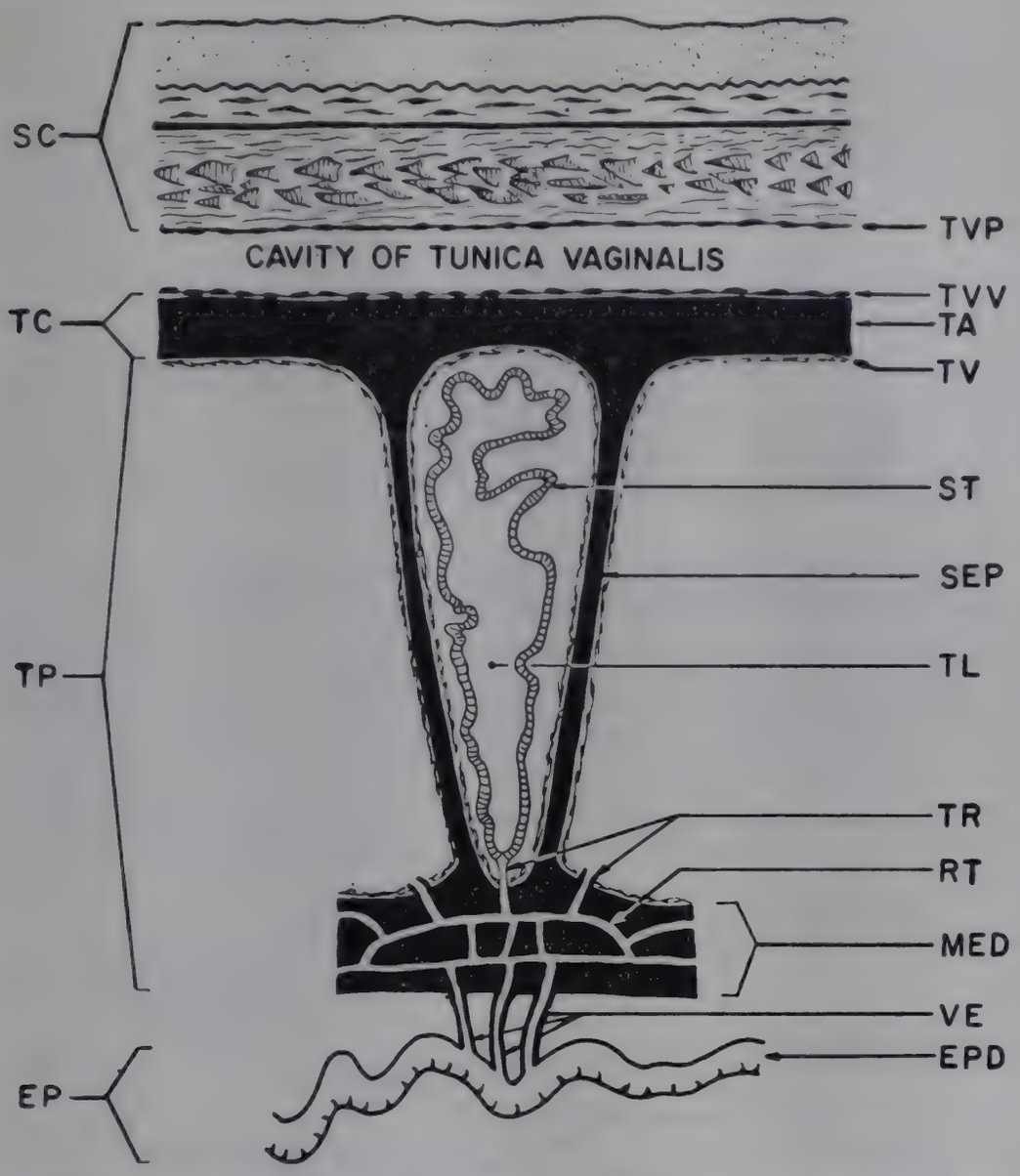


Figure 8. Diagrammatic section of the scrotum, testis and the epididymis (after Davis *et al.* 1970, modified)

with lymph channels which may be separated from the Leydig cells by loose connective tissue (Fawcett *et al.* 1969).

The outer layers referred to above, also called the boundary tissue, form a supportive framework and the myoid cells are rhythmically contractile (caused by prostaglandins ?) causing a peristaltic movement of the tubule to move non-motile spermatozoa (Roosen Runge 1951). Th antiandrogen cyproterone acetate suppresses the contractility of the myoid cells *in vitro* (Fabbrini *et al.* 1977). The myoid cell layer is supposed to serve in three ways : (i) in contractility, (ii) in fibrinogenesis, and (iii) as a barrier mechanism (Fabbrini *et al.* 1977). Hutson and Stocco (1981) found that the myoid cells were not merely supporting elements but might be involved in the regulation of Sertoli cells. Tung and Fritz (1980) during *in vitro* experiments did not testify this. Fabbrini *et al.* (1977) surmised that salts, water, gonadotropins and androgen passed from the intertubular lumen into

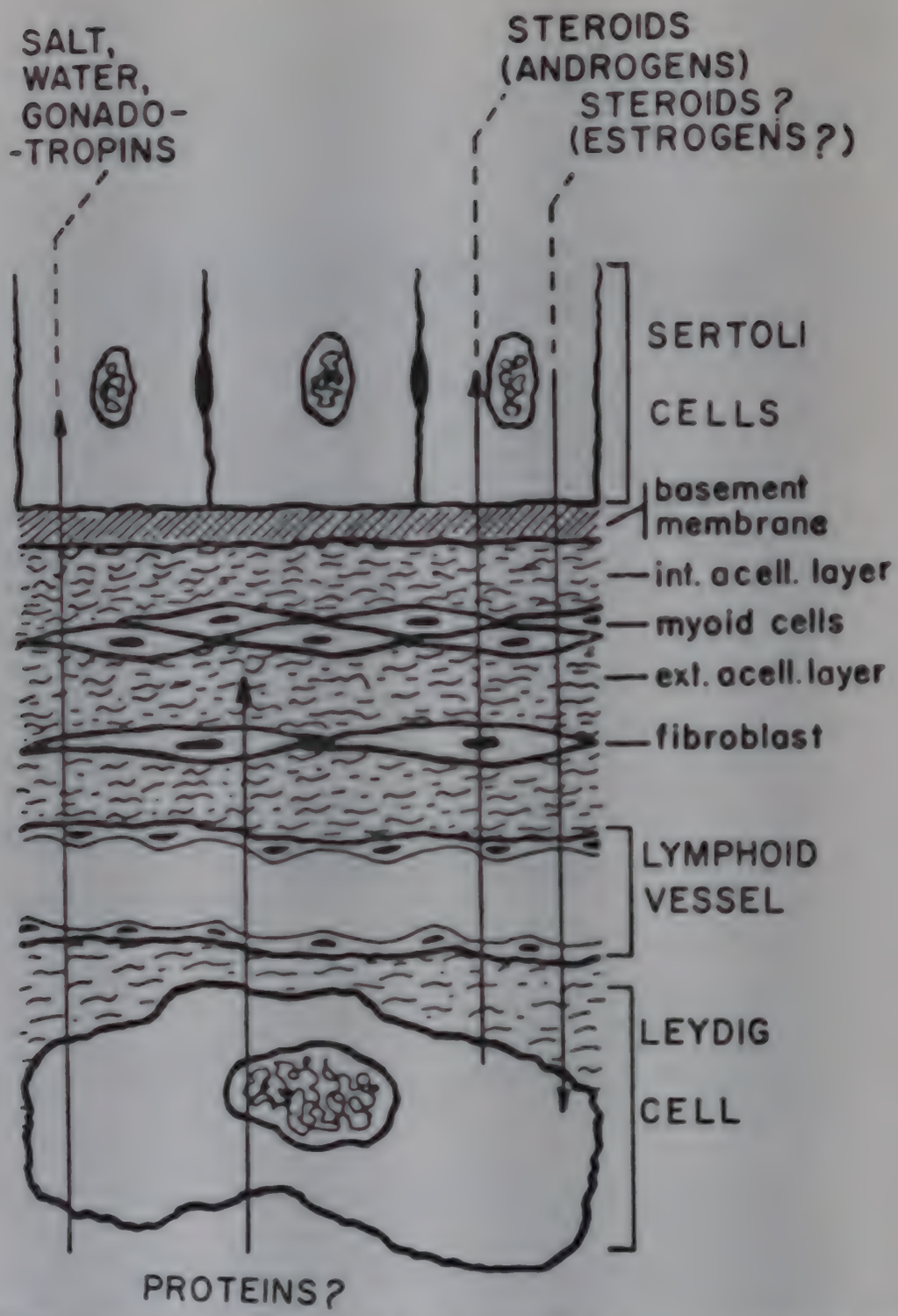


Figure 9. Diagrammatic representation of Sertoli cells, wall of tubulus, lymphatics and Leydig cell (after Fabbrini *et al.* 1977, modified)

the Sertoli cells while oestrogen from the Sertoli cells might pass in the opposite direction, as cytoplasmic and nuclear receptors are present in the Leydig cells.

Between the tubules, there are the interstitial or Leydig cells, blood vessels and lymph channels. In rat, according to Muller (1957), the tubules very rarely branch and do not communicate with one another. In other mammals, these may be connected at the tunica or along the length of the tubules.

Each end of the tubule towards the mediastinum gives rise to a tubulus rectus, having flattened cells, and the latter have been likened to the Sertoli cells by Roosen Runge (1961). Prakash *et al.* (1979, figure 2) observed a knob-like protrusion which they labelled as Sertoli cells. Before the tubulus rectus opens into the rete, its cells are reported to arrange themselves into a plug, functioning as a valve (Roosen Runge 1961); according to Dym (1974), this appearance is caused by the plane of sectioning.

In man, the septulae (extending from the tunica to mediastinum of the testis) divide the testis into about 250 pyramidal compartments called lobuli testis. Each lobule may contain 1–4 highly-coiled seminiferous tubules*, and the tubule of one may communicate with another in the next lobule. This highly coiled and convoluted appearance of the seminiferous tubules is assumed 17 weeks after fertilization in man and monkey (van Wagenen & Simpson 1965).

5b *The Exocrine-Secretory Function of the Seminiferous Epithelium*

This includes production and release of androgen-binding protein (*vide infra*) and secretion of a tubule fluid rich in potassium, glutamate and inositol (Bloom & Fawcett 1976), probably helpful in keeping the sperm alive. Ritzén *et al.* (1971) note that the seminiferous fluid shows high potassium and bicarbonate and low sodium and chloride contents as compared to blood plasma. This ionic gradient is maintained by the Sertoli cells and occluding junctions. The rete testis fluid secreted by its epithelium appears to be very little and its fluid component is got from the tubule. The fall in K^+ and testosterone and difference in protein concentration will have to be suitably explained. The two-fluid hypothesis may not be correct (Setchell 1978). The tubule fluid is not stationary and the flow does not appear to be controlled by any hormone. For the post-meiotic germ-cell maturation, the specific intratubular milieu, referred to above, is perhaps necessary.

5c *The Efferent Ducts*

These ducts show cuboidal cells and some may even show cilia. They also exhibit intercellular tight junctions at the luminal end in hamsters, rabbit man, etc. like those at the basal part of the Sertoli cells. In the human, at the upper part of the posterior edge of the testis some 12 efferent ductules arise from the rete and form vascular cones, as already said, opening into the epididymal duct at their bases. The tall cells alternating with low ones in the efferent ducts have cilia which move the spermia towards the epididymis (Bloom & Fawcett 1976).

6 THE KINETICS OF SPERMATOGENESIS

6a *Spermatogenesis*

The epithelial layer of tubules has two types of cells viz., the supporting or Sertoli cells and the germinal cells. The kinetics of spermatogenesis in the germ cells has been studied in a number of animals, very largely in the rat.

*Each seminiferous tuble is 30–70 cm long and their number varies between 800–1200 (Bloom & Fawcett 1976).

In a properly fixed and stained adult testis of a mammal (rat, monkey, man), the sections show spermatogonia, spermatocytes and spermatids; these names were coined by von La Valette St. George (1876). If a transverse section of the testis of rat (or rabbit, ram, bull, pig and some marsupials) and of man [or of the baboon, *Papio anubis* (Chowdhury & Steinberger 1976)] are compared, one notices a single cell or same cell (Setchell 1978) association throughout the section corresponding to stage VIII of the cycle (*vide infra*) in the animals listed above; in man, there may be four-cell associations corresponding to stages I, III, IV, and VI of the cycle (Clermont 1972) (or stages V, VI, VII of the baboon). Since the organization of the seminal epithelium of man resembles that of baboon (*Papio*), i.e., a patchlike appearance of cell associations (Chowdhury & Steinberger 1976), Dym's (1980) statement that 'the testes of apes and man may share a unique feature not encountered in other primates' may not be correct as baboon is not an ape but shows a mosaic arrangement of cell associations.

Two methods of classifying these associations are current among cytologists. One of them recognizes 8 stages; the other takes into account the features of acrosome and of the spermatids using periodic-Schiff reaction. In the rat, 19 steps are made out of the spermatids and since some cell associations have two generations, 14 such associations are recognized. In man, 6 associations and in the monkey

Figure 10. Diagram of a human seminiferous tubule showing germ cell associations (after Heller & Clermont 1964, modified)

12 associations are recognized. Figure 10 represents a human seminiferous tubule showing a mosaic of stages I–IV of the cycle (Heller & Clermont 1964).

To understand these stages of cycle of the seminiferous epithelium a reference may be made to figure 11 where XIV cellular associations or stages and the 19 steps by which a spermatid becomes modified or metamorphosed into a sperm (collectively known as spermiogenesis) are indicated (Perey *et al.* 1961).

6b *Concept of 'Cycle' and 'Wave' in the Seminiferous Tubule*

The *cycle* of the seminiferous epithelium is described as a 'series of changes in a given area of seminiferous epithelium between two appearances of the same developmental stages' (Leblond & Clermont 1952) or a dynamic histological phenomenon taking place with time in any one area of the seminiferous epithelium (Clermont 1972). Another term used is the *wave* of the seminiferous epithelium, and it refers to almost orderly distribution of cellular associations in the epithelium at any one time (figure 12). Regaud (1901) postulated that the 'wave is in space the cycle is in time'. Adjacent areas of the tubule are always at consecutive stages and in the rat and bull, the stages are arranged in 'wave' of complete sets of areas at consecutive stages. When one moves from rete, one does so from more advanced to less advanced stages. Pilsworth and Setchell (1981) describe each 'wave' as an open-ended cyst, a tubule forming a string of cysts with the tubule fluid flowing through the continuous cysts. It may also be described as the succession of all seminiferous epithelial cycle stages between two consecutive number 8 stages; the wave may be interrupted by a number of modulations or irregularities where local inversions in the stage succession do occur but not break its continuity (Perey *et al.* 1961). A quantitative analysis of germinal cell populations in man has shown that the 'wave' concept is not applicable and that the clone concept is more appropriate.

An examination of the cellular composition of the tubule discloses that there are spermatogonia, spermatocytes, spermatids and spermatozoa. Due to delayed cytokinesis, a clone of paired primary spermatocytes, of 4 secondary spermatocytes and of 8 spermatids appear, each giving a syncytial appearance as they show cytoplasmic bridges (Burgos *et al.* 1970). It is not clear when these break up. It is this syncytium which is the basis of synchronous differentiation where development proceeds independently of the surrounding cells and this is the basis of clonal concept referred to above. Sometimes two of these connected spermatids may not separate at all and one sees in the ejaculate double-headed abnormal sperm. The incomplete division of the spermatocytes and spermatids is so characteristic of the male germ cell that it demarcates them from the divisions of the somatic cells (Burgos *et al.* 1970).

Puberty sets in the male monkeys at 3–3.5 years but copulation is postponed (Conaway & Sade 1965); the first appearance of the spermatocyte in the monkey is about 3 years. In man, puberty sets in about 11–15 years (van Wagenen & Simpson 1965).

The other component of the tubule is the tall Sertoli cells which support the spermatocytes, spermatids and spermatozoa (sustentacular) (*vide infra*).

6c *Spermatogonia*

Regaud (1901) identified two types of spermatogonia in rats: (i) *dust-like*: nucleus

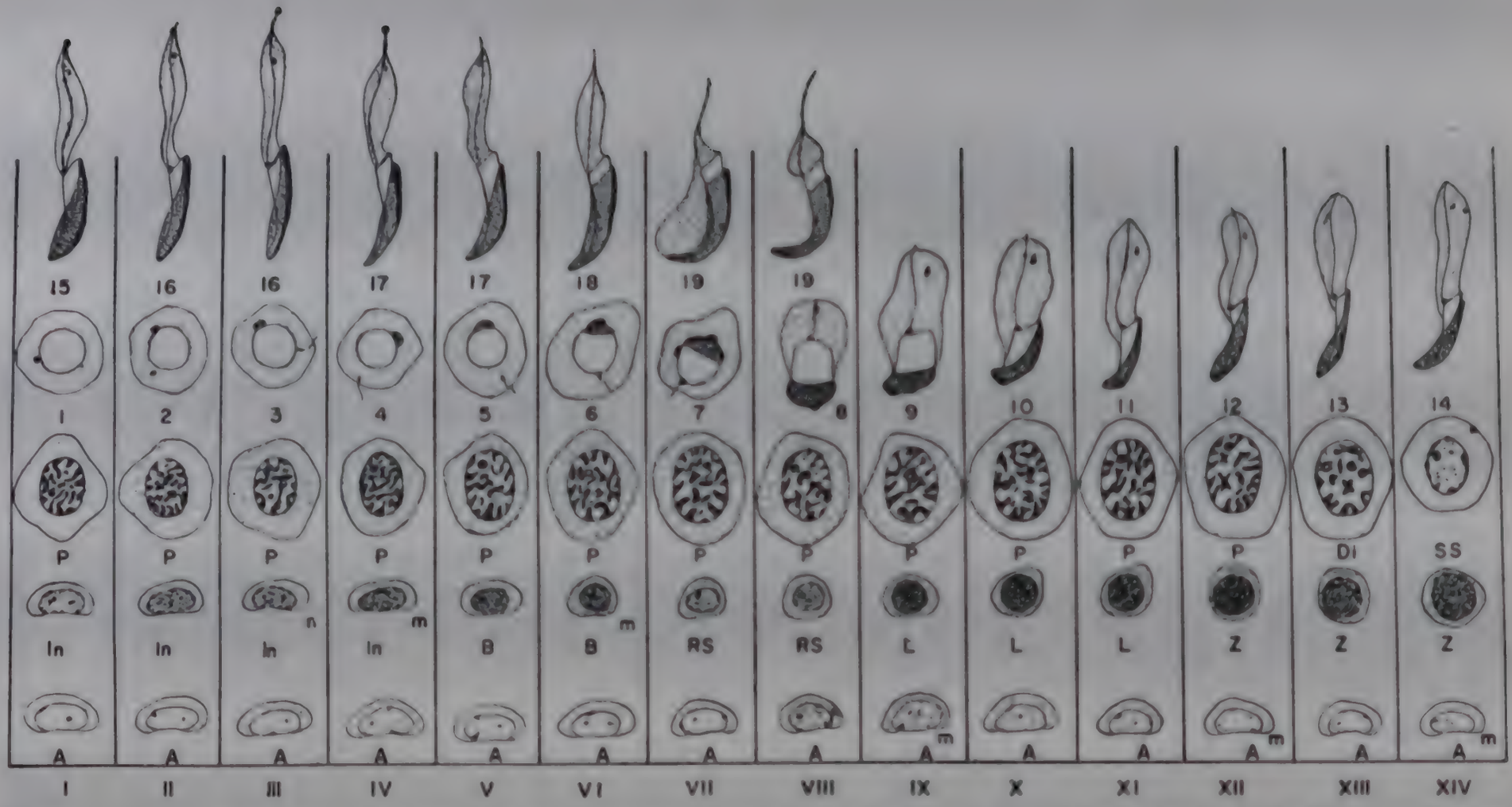


Figure 11. The 14 cellular associations seen in the seminiferous epithelium of the rat. They have been numbered I–XIV and each one depicts a cellular association or stage. As differentiation of the cells takes place the 14 associations succeed each other in time in known areas of the tubule and finally the association which was present in the area will appear in the same area. The succession in time of all associations in a given area of the seminiferous tubule represents the cycle of the epithelium. Spermiogenic cells include spermatids, steps 1–19 (after Perey *et al.* 1961, modified)

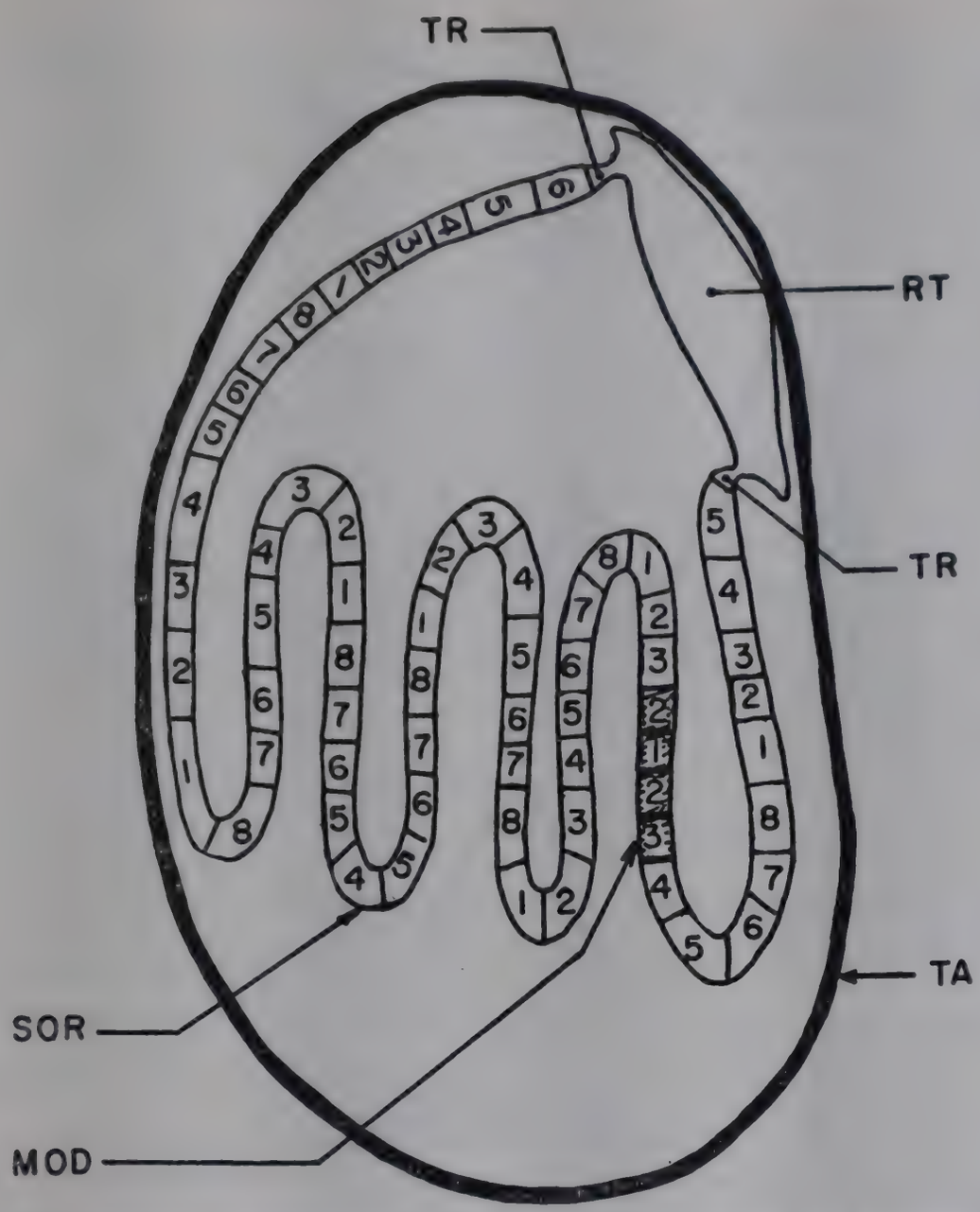


Figure 12. Diagrammatic representation of the stages of spermatogenesis in a tubule of the rat testis. Starting from the rete, the stages are arranged in descending order, forming 'waves' which proceed to a 'site of reversal'. Modulations or irregularities interrupt the wave (after Pilsworth & Setchell 1981, modified)

with fine and palely stained chromatin granulation—type A spermatogonia; and (ii) *crust-like* : presence of coarse granules or flakes of heavily-stained chromatin associated with nuclear membrane and nucleolus—type B spermatogonia. Such differentiation of spermatogonia into A and B types (Allen 1918) is characteristic of mammals like rodents; in the bull, monkey and man the differentiation is not that clear since in these the crusts of chromophilic chromatin is not very clear. Unless one uses a suitable fixative like Zenker-formol, the differentiation into A, B and intermediate types of spermatogonia becomes difficult to observe (figure 13). These spermatogonia, before giving rise to primary spermatocytes may pass through generations of crust-like spermatogonia and the first or the first two generations are

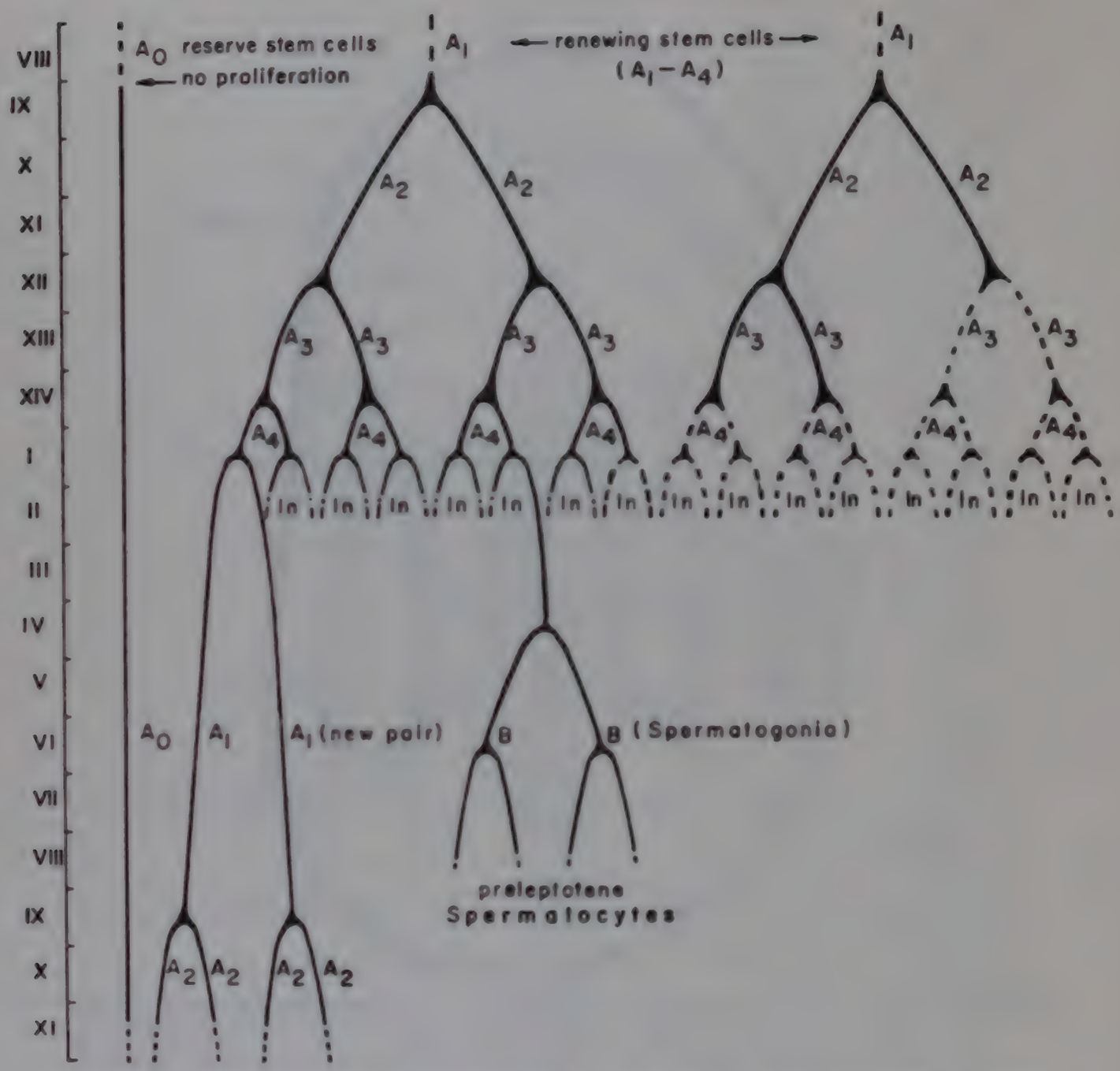


Figure 13. Model of renewal of spermatogonia in rat, studied in whole mounts. This suggests that the 'nodal' division of type A spermatogonia (A_4) was equivalent. A_0 does not divide and is a 'reserve stem cell'. As some A_2, A_3 and A_4 type of spermatogonia degenerate, intermediate type of spermatogonia are few, poduced by type A_1 spermatogonia, (after Clermont & Bustos-Obregon 1968)

called 'intermediate' type. While they have been described in the rat, mouse, etc., they are not found in man and monkey (Clermont & Leblond 1959). The type B spermatogonia may divide according to the species before giving rise tc the spermatocytes; in the monkey *Cercopithecus aethiops*. Clermont (1969) described four morphologically very similar successive generations of type B spermatogonia. Generally, the endoplasmic reticulum is poorly developed in the spermatogonia.

The type A spermatogonia sit right on the basement membrane while in the type B spermatogonium, the Sertoli cell cytoplasm may project incompletely, separating the Sertoli cell and the basement membrane (Courot *et al.* 1970).

Type B spermatogonia are the progenitors of the two generations of spermatocytes. The spermatocytes exhibit a definite arrangement in the Sertoli cells and are surrounded by Sertoli cell cytoplasm except at the region where the spermatocytes are connected by bridges forming a syncytium (Courot *et al.* 1970). The spermatocytes undergo meiotic division of the nucleus and give rise to the spermatids with haploid nucleus. The primary spermatocytes have a tetraploid nucleus while the secondary ones have a diploid one (Courot *et al.* 1970). The primary spermatocyte nuclei during interphase were described as 'resting', but as these are synthesizing DNA, Tobias (1956) pointed out the anomaly and called them the preleptotene spermatocytes and this terminology is widely used. The preleptotene spermatocyte nucleus closely resembles the type-B spermatogonial nucleus. After meiotic reduction of chromosomes in the primary spermatocyte showing tetraploid nucleus (Courot *et al.* 1970), the secondary spermatocyte stage showing diploid nucleus is reached and it undergoes mitosis (second maturation division) and gives rise to spermatids with haploid number of chromosomes. Typical non-occluding gap-junctions and cytoplasmic processes entering deep into the Sertoli cells anchor the non-motile spermatids loosely by their head ends (figure 17), to the Sertoli cells. The spermatids undergo phenomenal changes before becoming spermatozoa. When the spermatozoa escape into the luminal fluid, they leave behind their cytoplasm, now called the residual body (figure 33) and this may be phagocytosed by the Sertoli cell; a small part of the residual body may also escape into the tubular lumen, while a very small cytoplasmic droplet goes with the sperm.

A question arises as to what happens to the type A spermatogonia. In understanding this and without going into the descriptions of previous workers [particularly of Clermont & Leblond (1953) who proposed a 'stem-cell renewal theory' not generally accepted], Clermont and Bustos-Obregon (1968) examined the stained tubule cells *in situ*. Two main lines of type A spermatogonia were observed (figure 13). The type A_0 spermatogonium which never divided and therefore had no descendants, was called a reserve stem cell. The group of A cells called renewing stem cells showed 4 groups of cells appearing in succession. In the rat where the cycle (from spermatogonium to sperm) can be divided into XIV stages (figure 11), the origin of renewing stem cells is programmed as follows:

1. A_1 cells divide at stage IX to yield A_2 cells;
2. A_2 cells divide at stage XII to yield A_3 cells;
3. A_3 cells divide at stage XIV to yield A_4 spermatogonia.

A_4 spermatogonia divide at stage I to give rise to intermediate cells and new type of A_1 cells. The intermediate spermatogonia produce B cells at stage IV. These B cells give rise to preleptotene spermatocytes in stage VI and the type A_1 cells after an interphase restart the divisional series in stage IX and the cycle goes on.

According to Kramer *et al.* (1964) the spermatogonia could behave in a variable manner and their co-ordination is probably under the control of the Sertoli cell.

6d *Hormones and Spermatogenesis; Chalones*

Steinberger (1971) brought out that gonocytes multiply prenatally and a few days after birth, the formation of primitive spermatogonia A is probably influenced by testosterone (figure 14); formation of types A and B spermatogonia, primary spermatocytes and resting, lepto-, zygo- and pachytene spermatocytes is not supported by testosterone or gonadotropic hormone; it may be influenced by growth hormone. Reduction division appears to be controlled by testosterone. Up to steps I to XV formation, no hormone is needed while from XVI to XIX, FSH is necessary. The role of these hormones during spermatogenesis has been brought out somewhat differently by Lostroh (1976) (figure 15). Spermatogonia A and B and primary spermatocyte (meiotic prophase) do not require any hormone-inducement (figure 15) and develop independently. For the passage of primary spermatocyte (prophase) into metaphase and then into the secondary spermatocyte, testosterone is necessary; for the formation of spermatid, moderate amount of gonadotropins is necessary; immature spermatozoa can be formed if there is abundance of FSH and LH; for the maturation of spermatozoa, testosterone is necessary. It is believed that the two gonadotropins act synergically. Hansson *et al.* (1976) in summarising the previous work on the relationship between hormones and spermatogenesis, brought out that the latter needs active Sertoli cells and androgen. FSH alone can maintain Sertoli cell function in hypophysectomised animals and it cannot stimulate spermatogenesis in the absence of androgen. Androgen can maintain both Sertoli cell function and spermatogenesis. It is known that in hypophysectomised rat, T can maintain spermatogenesis. Other C^{19} steroids appear to lend a helping hand in this. Further work by Ludwig (1950) brought out that the damage caused to seminal epithelium by low doses of T is secondary to gonadotropin suppression; he also noted that maintenance of sperma-

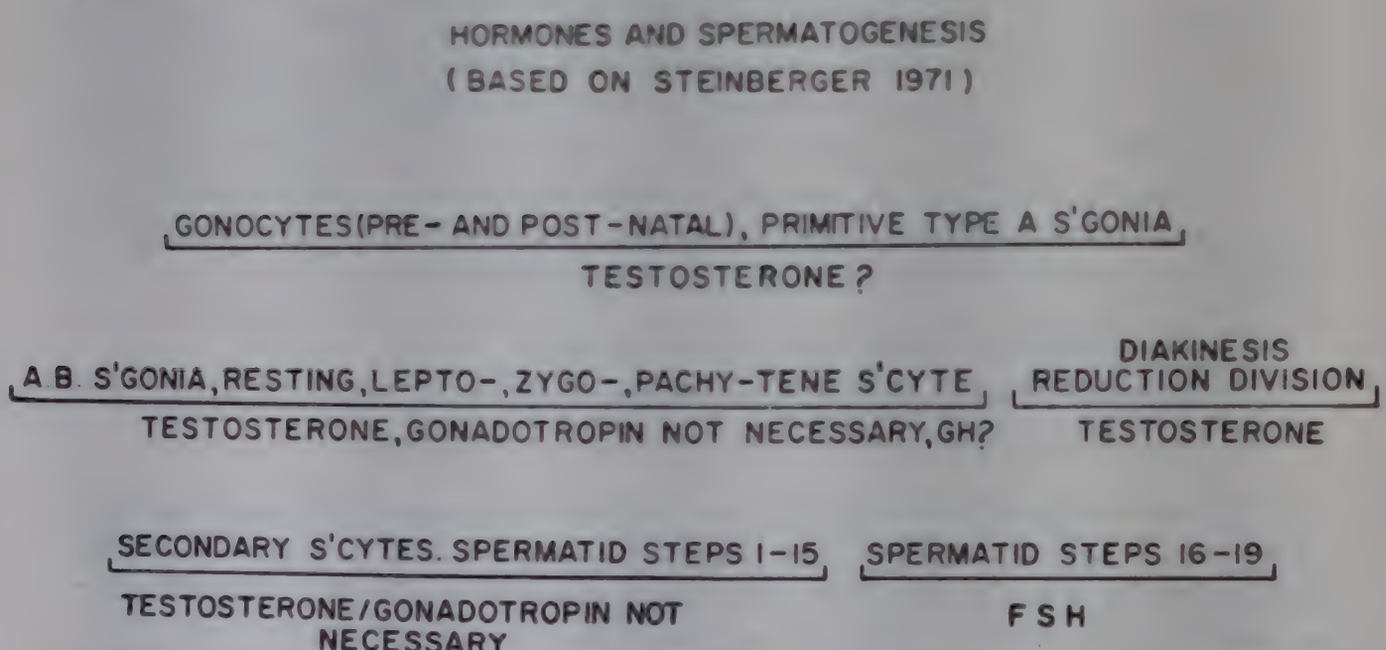


Figure 14. The effect of testosterone and gonadotropins on the different phases of spermatogenesis (after Steinberger 1971, modified)

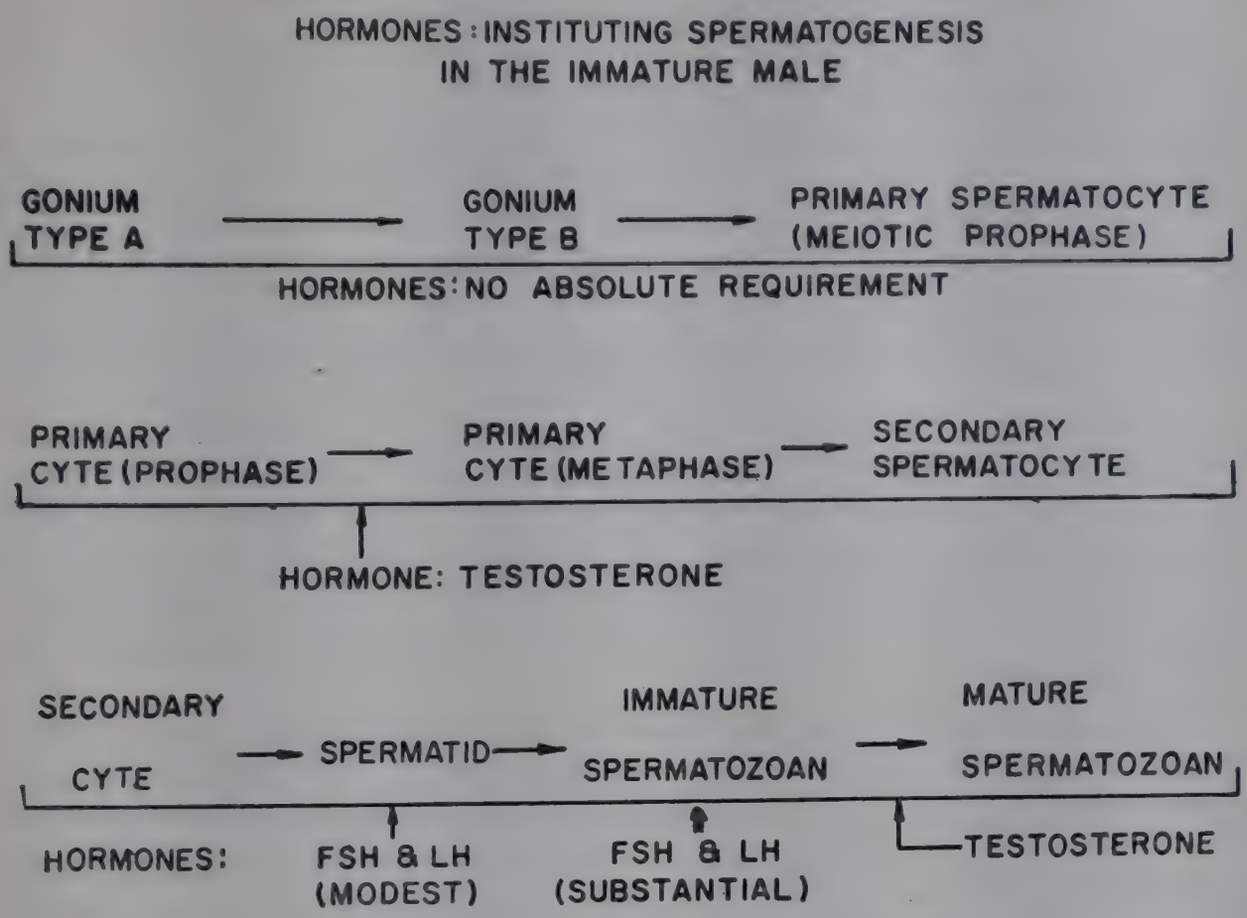


Figure 15. The effect of testosterone and gonadotropins on the different phases of spermatogenesis (after Lostroh 1976, modified)

togenesis by high doses of T is secondary to direct stimulating effect of steroid on spermatogenesis occurring in spite of reduced gonadotropin levels.

Androgen and FSH can maintain and initiate spermatogenesis. Adult rats do not need FSH to maintain spermatogenesis (Dym *et al.* 1979). In man, even when the plasma FSH is so low that RIA cannot detect it, spermatogenesis is maintained (Sherins 1974). Administration of anti-FSH serum to male bonnet monkeys for 21 days, reduced the percentage of live spermatozoa, of the total sperm count and fertility and therefore it was concluded that FSH was necessary (Murty *et al.* 1979), In ground squirrels and monkeys (Wells 1942, Smith 1944) T can maintain spermatogenesis in hypophysectomised animals even though gonadotropins are absent and when the testes regress, it can reinitiate spermatogenesis.

Chalones: Clermont and Mauger (1974) brought out that in A spermatogonia of irradiated adult or 33 day-old rats, a substance called chalone in the testis lowered the incorporation of tritiated thymidine. This substance in normal conditions may control the mitotic rate of these cells and is present in cells of many tissues having local effect on cell division.

6e *Cyclic Variation of Steroidal-output in Primates*

While the reproductive biology of the female primate has engaged the attention of workers from a long time, that of the male has been sadly neglected. This is largely

because spermatogenesis in the male is restricted to a period of four months in the best studied monkey, the rhesus, according to Conaway and Sade (1968) and during the remaining period,—the testes are either regressing if not already regressed. In addition, the male is proverbially aggressive and advisedly, it had to be kept at a distance. Now it is apparent that in studying fertility-control in man, comparative knowledge of the male monkey reproductive pattern is becoming more and more important since drugs useful in controlling male-fertility may have to be first tested on monkeys. Paradoxically, in the owl monkey *Aotus* (Dixson *et al.* 1980), the testes normally show a regressed condition (due probably to vitamin E-deficiency) even in males that are breeding successfully under laboratory condition .

We have in India five species of *Macaca* (the rhesus, the stumptail, the bonnet, the Assamese and the lion-tail), five species of *Presbytis* (the hanuman, the Nilgiri, the golden, the spectacled and the capped langurs), two genera of prosimia (omitting the insectivorous shrews) viz., the slender and slow lorises, and an anthropoid ape (the gibbon). Some baseline data after investigating these primates have revealed a number of interesting similarities to man and, therefore, more work is called for.

In the male, the diurnal or circadian (circa — approximate, diem — daily) rhythm concerns the two testicular steroid hormones viz., testosterone (T) and its reduced form, i.e., 5α-dihydrotestosterone (5α-DHT); the adrenal steroid cortisol also exhibits a diurnal rhythm. In man, there is a daily (circadian), an annual (circannual) and also a pulsatile (circhoral) hormone rhythm (Ewing *et al.* 1980). Circadian and circannual rhythms have been described in a number of monkeys.

There are at least six functions attributed to testosterone in mammals and the hormone shows a surge in its diurnal rhythm. Peculiarly the peak of T in man is reached in the morning, while in the male monkey it is during the night and maintained till the next morning. These two peek values have been experimentally proved to be not due to poor metabolization, or clearance rate or excretion. It would be exceedingly interesting to know the functional importance of the presence or absence of daily T rhythms, since our knowledge in this regard is very little.

Interestingly enough, in the free ranging colonies of rhesus monkeys (e.g. Cayo Santiago, Peurto Rico) where environment plays a major role in reproduction and in the laboratory where environmental influences are completely eliminated and where the temperature and relative periods of light and darkness (LD 12: 12) are controlled, the reproductive rhythm was the same (Michael & Keverne 1971). These authors were primarily concerned with the study of ejaculatory performance in rhesus males and they found that it was maximum during December, i.e., during the height of reproductive season. They concluded that the various rhythms that the male animal manifested in its reproductive life were due to the physiological activity of the testis and were innate to it or genetic. On the other hand, Vandenberg (1973) opined that mating, etc, was due to sequential environmental factors.

A host of workers has brought out that the primates exhibit a circadian variation of blood T, the peak being reached during the night and maintained till next morning (Dray *et al.* 1965). This diurnal variation can be removed by injecting hCG into the male (Nieschlag *et al.* 1971). Michael *et al.* (1974) studied the plasma T and cortico-

steroids using unanaesthesized rhesus male monkeys and collected blood by vena puncture at 4-hr intervals; they found a peak at 2200 hr; in the castrate monkey, this peak was abolished and there was a contribution of T by the adrenal. Rose *et al.* (1978) noticed that in rhesus monkeys living in social groups, the males, interacting with sexually active females, showed a two-fold increase in T. The T level came down to basal levels when the females were taken away. They described a diurnal rhythm not only in T-concentration but also in cortisol level, both reaching the peak values at the same time. Goodman *et al.* (1974) studying T levels in rhesus adult males noticed the diurnal rhythms in normals and in orchidectomised males, there was also an elevation of LH and it is not clear if this rise is the cause of T surge: however, see below.

Very convincing evidence of the diurnal variation in the T level of the male and female rhesus monkeys has been provided by Perachio *et al.* (1977). Gonadectomised animals were also studied. The monkeys were subjected to least stress as they were chair-adapted and catheterised; awakening the animals for taking out blood was thus avoided. In the intact males, there was a diurnal variation with a marked increase in the early evening (1900–2200 hr) as compared to day levels and this rise was retained throughout the dark period. In the castrate there was a nadir during the early evening (2000–2200 hr). This evening nadir of T was 56% lower than that of the 24 hr mean, while the maximum serum level was 46.4% higher. Obviously, there are two circadian rhythms—one in the intact animal and the other in the castrate. In the intact and ovariectomised females, the T values were higher during the day and showed a nadir during early evening. The adrenal androgen in an orchidectomised male also showed a diurnal circadian pattern characterised by a nadir in the evening; this adrenal pattern is eclipsed by the larger gonadal rhythm in the intact male.

Anand Kumar *et al.* (1980) have studied the circadian rhythms in the male rhesus monkeys maintained in their colony under controlled conditions. They described that the T peak was during the night (12 PM) rising from 4 ng level to 16 ng/ml. During the two months, January and July, when they studied serum T levels, they found even less than 8 ng/ml of T concentration during January. Their finding of rhesus males having high concentration of T in July is rather peculiar and needs re-examination utilizing more number of monkeys and see if LH also shows high values (Nieschlag & Wickings 1980).

In another experiment, Puri *et al.* (1980) maintained 15 male intact rhesus monkeys under animal husbandry conditions LD 12 : 12. Venous blood and cisternal cerebrospinal fluid (CSF) were collected once between 9 and 11 AM and again between 21 and 23 PM. All the 15 monkeys showed the presence of T, cortisol (C), prolactin (PRL) and biologically-active LH (bLH) in the CSF; however, there was considerable variation in these hormones among the individuals probably due to intrinsic differences among the animals. Serum levels of these hormones showed a nycthemeral rhythm in the rhesus monkey. Serum T and PRL levels were *higher* during the night than during the morning. There were a few rhesus monkeys which did not show this pattern at all. LH does not appear to show a nycthemeral rhythm studied by RIA methods. A study of the ratio between serum and CSF brought out for T (53 in day samples and 102 in night samples) shows that the

serum: CSF ratio for C, PRL and bLH was below 20 and there was not much difference between the day and night samples. This is probably because the transfer of T into CSF is very little after i.v. injection, compared with other hormones.

Seasonal variations: Wickings and Nieschlag (1977) imported rhesus monkeys from India into Germany and they maintained them in animal houses with strict husbandry conditions in which the primates were excluded from all environmental impacts. It was exclusively a male colony. They made 11 important observations and three conclusions :

(*i*) DHT appears 4 weeks later than T and indicates when exactly 5α-reductase system starts working;

(*ii*) The inverse pattern of dehydroepiandrosterone (DHEA) and Prolactin to that of LH and T speaks of the involvement of adrenal and of prolactin in testicular function, and the

(*iii*) Peak of FSH noticed during June probably triggers spermatogenesis and higher values of FSH during regression speak of resetting of feedback mechanism.

All these have led the two authors to postulate the existence of an inherent circannual regulating mechanism synchronising testicular functions in monkeys.

In the south Indian bonnet monkeys (*M. radiata*), Mukku *et al.* (1976) have shown the existence of a diurnal variation in T; they also showed that in the laboratory, continuous illumination caused the nycthemeral variation in T to disappear and it reappeared if the 12 : 12 LD regimen was brought back.

In the male langur monkey (*Presbytis entellus*), reproductively active throughout the year, there is a clear diurnal variation in T (10.49 n mol/litre in the night and 6.48 n mol/litre during day) and there is no reference to circannual variation (Lohia *et al.* 1982). There were no circannual variations in seminal characteristics. According to Chowdhury *et al.* (1980) there is a circannual variation of plasma testosterone in the male langur monkey (*Presbytis entellus*), resembling the bonnet (Kamboj *et al.* 1982) and the rhesus.

In the "Report of the work done for the years 1978-79, (Institute of Research for Reproduction, Bombay)", it has been reported that there is no seasonal or circannual variation of T concentration or in semen volume or sperm density in the bonnet monkeys. Munshi (1980) also reported that there was no circannual pattern of T levels in the bonnet monkeys. Kamboj *et al.* (1982), on the other hand, have shown the presence of diurnal variations in plasma T levels (reaching a peak at 2100 hr) and also a seasonal variation in bonnet monkeys maintained under strict animal husbandry conditions. It reached a peak during the breeding months (September and October); Munshi also showed peak values during August and November in one of the four monkeys used for the study but affirmed that there was no circannual pattern. Murty *et al.* (1979) recommended the bonnet monkey as a better model than rhesus for contraceptive drug study as it did not show a seasonal variation in sperm number or serum T concentration throughout the year. Jayaraman *et al.* (1980) did not notice any visible sign of testis size change or failure of ejaculation in bonnets.

Semen content and sperm number in the bonnets and baboons appear to show no change seasonally (Sharma *et al.* 1982).

In a behavioural study using the West African talapoin monkey (*Cercopithecus talapoin*), Hansen *et al.* (1980) noted that in the most dominant and the most subordinate males, when isolated from females of the colony, the LH and T levels were very much the same (however, the prolactin level was higher in the subordinate males). When both dominant and subordinate males were housed with sexually attractive females, the LH and T levels increased only in the dominant males; if the dominant males were removed, the subordinate males showed increased level of LH and T (see also p. 23, bottom).

As regards the correlation between LH and T peaks noticed even in man (Naftolin *et al.* 1973), there have been a number of investigations and in all these, the total testosterone content has been taken for correlation. They found no such correlation between LH and T (Alford *et al.* 1973a, 1973b) and there was a pulsatile or circhoral secretion of both pituitary and gonadal hormones. Steinberger *et al.* (1977) reported that the 'poor correlation between T and LH spikes could indicate that the moment-to-moment pulsatory gonadotropin secretion is independent of gonadal feedback'.

With regard to the prosimians *Galago* and *Lemur,* only the latter showed a nadir and two peaks during night (van Horn *et al.* 1976). More work is clearly indicated.

Not only the adult testis but also fetal testis can secrete T in monkeys (Resko 1970, Resko *et al.* 1973). In rhesus fetuses of 46 and 60 days, the testis can convert ^{14}C-pregnenolone into T and androstenedione *in vitro.* In fetuses of 100, 125 or 150 days, the male umbilical artery showed more of T than in a female fetus; while fetal testis synthesized and released T into fetal circulation, the fetal ovary was relatively quiet. Castration of male fetus on day 100 of gestation stopped T secretion found on 150–156 days of gestation (Resko *et al.* 1973). This is sufficient indication to show that the fetal males develop in a different hormonal milieu than the female fetuses.

The neonatal rhesus of both sexes were examined for the first 14 weeks for T, DHT, oestradiol and cortisol levels. Some castrates were also studied (Robinson & Bridson 1978). Male and female pigtail monkeys (*M. nemestrina*) were also examined. They found that the neonates (male and female) showed the presence of T and DHT in different proportions and after castration, the level was very low. The pigtail (male and female) neonatals also showed the presence of T in different proportions. With regard to cortisol in the rhesus neonates, in the first two postnatal weeks, T concentration fell to adult levels and then differences between male and female levels were not statistically significant as also the oestradiol levels in both sexes of the neonates.

It has been brought out by workers on behavioural studies that in the non-human primates there is a positive correlation among the three features viz., aggressiveness, social rank and plasma concentration of T in both male and female monkeys (Kling 1975). Aggressiveness is considered an adaptive mechanism for passing genetic material of survival value. Circulation of fetal androgen influences the brain and this determines phenotypic-gender-related behavioural

patterns. Goy (1970) brought out that genetic females exposed to high levels of androgens behave like genetic males in their pattern of play and assertiveness. We have also recorded (Ramaswami & Anand Kumar 1962) that the adult female *Loris* receiving a total dose of 4.5 mg of testosterone propionate (Ciba) showed great aggressiveness when one approached it, a feature not generally noticed in normal females.

While I have described at length the male rhythms in Primates, an interesting feature noticed in the insectivore *Suncus* regarding androgen rhythm is worth repitition. It was reported (Dryden & McAllister 1970) that spermatogenesis in the nonscrotal testis but enclosed in cremaster sacs of the shrew *Suncus murinus* was unaffected by cadmium salt. Following this observation, testicular development and testosterone concentrations in the young male shrew were studied by Hasler *et al.*(1975); they included castrated males also in their hormonal studies. Both testis development and plasma testosterone showed two peak values by about 30 and 45 days respectively; the spermatid maturation took place during the first peak when the testes had reached their maximum weight, and during the second, spermatozoa were plentiful in the epididymis. After the first peak period, some of the tubules appeared 'empty' except for a few spermatogonia and Sertoli cell-embedded spermatids. Mathur and Goyal (1972) studying the Indian *Suncus* reported that the tubules showing spermatids did not disclose any other cell type; such tubules were not, however, met with by Hasler *et al.* (1975) in the same species of shrews studied by them.

6f *Radioautography*

Radioautography has helped in understanding the duration of cycle of the seminiferous epithelium in a mammal; in the mouse it is 8.6 days (Oakberg 1956) and in man, it is 16 days (Heller & Clermont 1964). The duration of spermatogenesis is 34.4 days (4 times 8.6) in the mouse and 64 days in man. Courot *et al.* (1970) described the 64-days cycle as 'amputated spermatogenesis' as it comprised 4 cycles of seminiferous epithelial cycle only. Man has the longest seminiferous epithelial cycle (16 days) while the boar has the shortest (8.0–8.6 days) indicating that four cycles constitute complete spermatogenesis. Clermont (1972) felt that the development of the various generations of germ cells and the maintenance of their characteristic associations were controlled by Sertoli cells which, however, needs further study.

As the germ cells progress in the kinetics of spermatogenesis and become differentiated into spermatozoa they move towards the lumen of the seminiferous tubule. This phenomenon can only be appreciated when one knows something about the supporting or Sertoli cells, their architecture and biochemistry.

7 THE SERTOLI CELL (figures 16, 18 and 19)

In 1865, the Italian scientist Sertoli described the cell, which bears his name, after making dissections of fixed testis material as microtomy was unknown then. This was considered a 'nurse' cell; in fact the precursor of the adult Sertoli cell was called 'nurse' or 'supporting' cell. These cells were thought to be in the nature of syncytium and this polemic was finally abated that they are not syncytial (Gondos 1977).

7a *The Differentiation of the Sertoli Cell*

The differentiation of the gonadal primordium into a testis and the maturation of the Sertoli cells go hand in hand (Ritzen *et al.* 1981). The indispensability of a cell-surface component—the H-Y antigen (the histocompatibility antigen depending on Y sex-chromosome) for testicular development was experimentally proved by Wachtel *et al.* (1977). It has been further shown by Mueller *et al.* (1978) that in the male rat, H-Y antigen is actively secreted by the gonadal tissue; the release of the antigen can be prevented by cyclohexamide. The H-Y antigen also moves into the epididymes and increases as age advances. Further, Wolf (1979) studied 12 human XY gonadal dysgenesis patients who happened to be genetically heterogeneous. According to him, the negative cases or those with reduced antigen titre, the H-Y-generating system is influenced by mutation. In the positive ones, the target cells are unable to react due to a defective gonad-specific H-Y antigen receptor. Sharat Chandra (1983) has reviewed the available information regarding testis differentiation in mammals. He also appears to support the 'basic femaleness' hypothesis of the mammalian embryo enunciated by Weissner (1934, 1935). If, however, the gonadal primordium has a Y-chromosome, then it tends to be a male. Both the chromosomes (X & Y) have been mapped; in the male the X shows more genes than any other chromosome which may be due to it being present hemizygously, and peculiarly, the Y chromosome does not show even a single Mendelian gene with male determining properties though the chromosome is present hemizygously. Further, the H-Y antigen and the serologically-detectable male antigen (SDM) were till recently thought to be one and the same and played a major role in testis-differentiation. Recent work (Silvers *et al.* 1982) has brought out that the above two genes are very different and have nothing to do with male differentiation. In the wood-lemming *Myopus*, Winking *et al.* (1981) found XY-chromosome constitution in fertile females. Studying hybrids of *musculus* and *poschiavinus* species of *Mus*, Eicher (1982) showed the existence of a male determining gene (*Tdy*) on the Y chromosome.

In the presence of the H-Y antigen, mesenchymatous medullary gonadal blastema cells differentiate into primordial Sertoli cells. These form cell cords or sex cords into which the primordial endodermal germ cells (migrating from the yolk-sac) enter. That the Sertoli cells may have a dual origin, viz., from the coelomic epithelium and the mesenchyme has not been accepted by all; Jost *et al.* (1974) are inclined to the mesenchymatous origin of the Sertoli cells. Gondos (1977) brought out that ultrastructurally they seem to resemble the surface epithelial cells and not mesenchyme ones.

The Sertoli cells in the adult are non-proliferative and divide mitotically only in young animal (first week postnatally in the rat). However, in the lower vertebrates, Stanley (1966) in Elasmobranchii and Billard (1969) in *Poecilia* have shown that the Sertoli cells divide in the adult at the beginning of each spermatogenic series This is so in all vertebrates where the testes are patterned as cysts.

As Bloom and Fawcett (1976) pointed out, the main functions of the Sertoli cells are mechanical support and protection of the germinal cells That they are nutritive has still to be established.

It has been reported that even high dose of radiation (Rugh 1952) does not affect the Sertoli cells; even embryonic precursors (when the pregnant mother is irradiated) are radio-resistant. The possibility of ultrastructural damage or biochemical damage or loss of physiological function cannot be ruled out. The number of Sertoli cells may be halved by irradiation in immature animals (Erickson & Blend 1976). According to Bateman (1958) it is likely that irradiation causes 'ageing' effect and the cell accumulates lipid material.

The Sertoli cells include within their intercellular spaces proliferating germ cells (except the spermatogonia, all other germ cells) which are surrounded by Sertoli cell processes and one is driven to infer that the Sertoli cell is the obligatory anatomical pathway between germ cells and blood (Burgos *et al.* 1970). The proliferating clone of germ cells being syncytial, led Roosen Runge (1962) and others to consider the association of Sertoli cells and germ cells as symbiotic; organelle-rich Sertoli cells probably help germ cells poor in them.

Johnsen (1969) showed the existence of two types of human testicular Sertoli cells, based on the staining properties of the nucleus using the classical Hiedenhein's iron-haematoxylin method. In type A cell, the nucleus is not stained while in type B, it is intensely stained. In Sertoli-cell-only syndrome, both the types are easily met with while in normal spermatogenesis, type B is very scarce. Both types are met with in infantile testis, in hypophysectomised and also in Sertoli-cell-only human testis. Fawcett (1977) stated that the description of double Sertoli cell types based either on endoplasmic reticulum or on nuclei 'merely reflect different phases of physiological activity of a single cell type'.

7b *Cytoplasm of Sertoli Cells*

The mitochondria (figure 16) are slender and along with transverse tubular cristae; their orientation is different in the basal and supranuclear cytoplasm. In the former, they are randomly distributed while in the latter, they are parallel with the longitudinal cell axis.

In the Golgi complex multiple separate Golgi elements are present in the basal cytoplasm while a few are also found in the supranuclear one.

Lysosomes are also present and these are supposed to digest the degenerating germ cells and the residual bodies left behind in the Sertoli cell when the sperms disengage themselves from the Sertoli cell (Reddy & Svoboda 1967).

Both types of endoplasmic reticulum (ER) are present in the Sertoli cell. The granular ER is noticed largely in the basal cytoplasm (usually in the form of tubules) and the smooth ER is noticed to a greater extent than the rough; there are, however, species differences among the mammals. Dense accumulation of smooth ER (figure 16) surrounding the developing acrosome of the spermatid is noticed. It has been found that the smooth ER is more involved in metabolism of testosterone than its synthesis (Ritzén *et al.* 1981).

The cytoplasm of the Sertoli cell may show large (10–25 μ) and small (1–5 μ) crystalloids; ultrastructurally, they answer to microtubules (Burgos *et al.* 1970). Lipid may also be present but it varies according to the species. Lacy (1967) brought out that X-irradiation or oestrogen administration caused the degeneration of germ cell; *pari passu* with this the lipid content went up considerably in the Sertoli

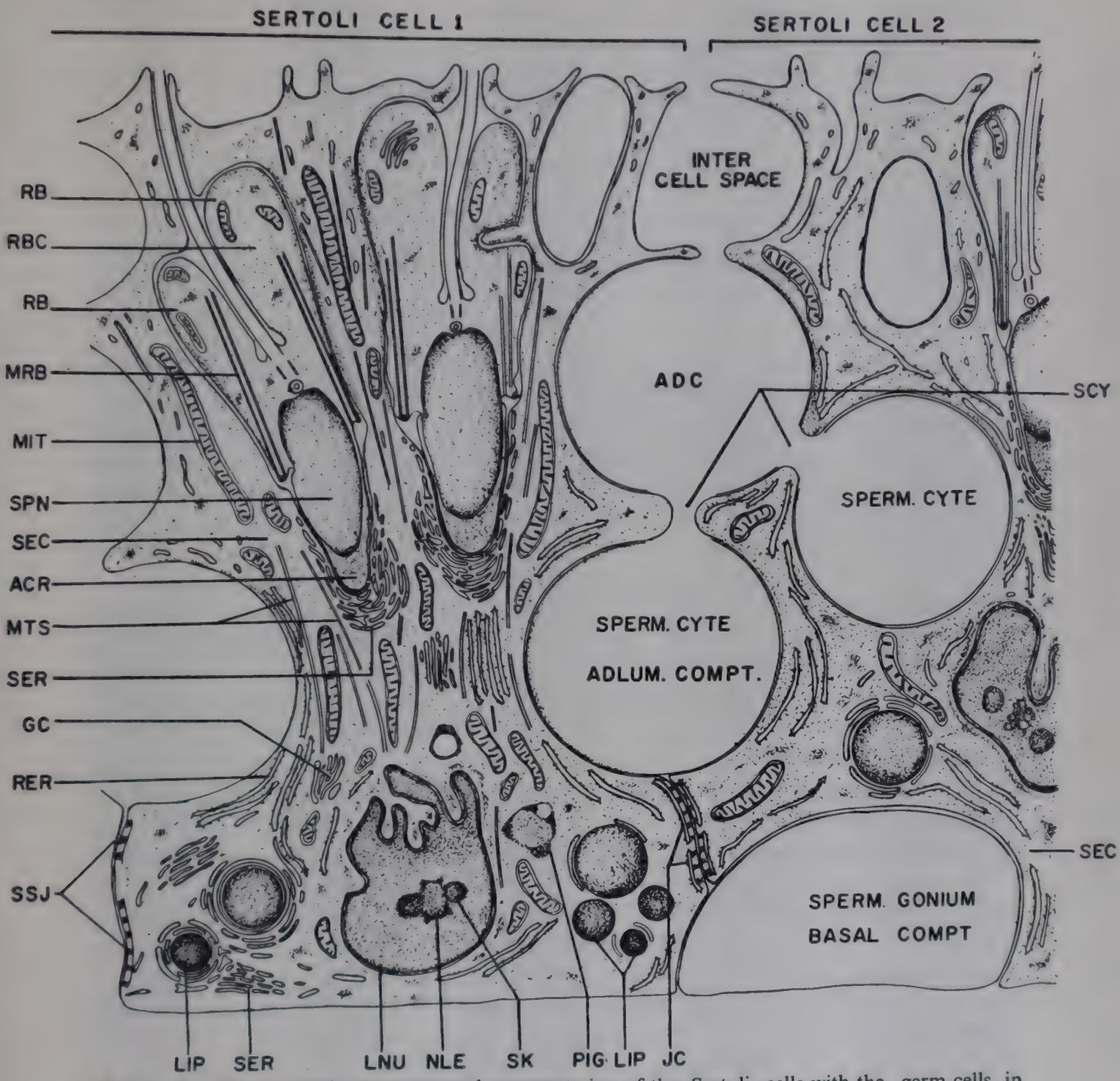


Figure 16. Diagrammatic ultrastructural representation of the Sertoli cells with the germ cells in various stages of growth, the blood-testis barrier and the lobed nucleus (after Fawcett 1975, modified)

cell. He even proposed that the residual bodies ingested by the Sertoli cell would form the raw material for steroid synthesis and these local hormones brought about spermatocyte meiosis and a further crop of spermatids. However, recent studies have not confirmed the *de novo* synthesis of oestrogen (as also steroids in general, Dorrington *et al.* 1978a) even though the Sertoli cell has all the complements for it. In cultured immature rat Sertoli cells (though Steinberger was sceptical

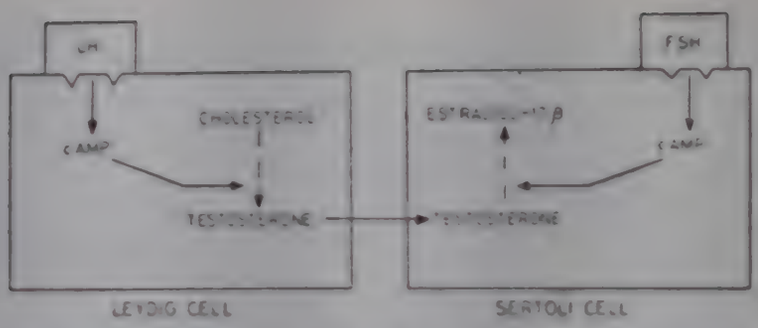


Figure 17. The two cell-two gonadotropin hypothesis (after Dorrington *et al.* 1976, modified)

of getting pure culture of Sertoli cells), aromatization (the conversion of 19-hydroxylated androgens to oestrogens, Dorrington *et al.* 1978b) of exogenous T to oestradiol (augmented by FSH administration or by addition of large doses of dibutyryl CAMP (an analogue of CAMP, mimicing LH) was reported by Dorrington *et al.* (1975). The same idea was brought out in a model of two-cell two-gonadotropin hypothesis (Dorrington *et al.* 1976, 1978b) (figure 17) where it acts as a messenger system between the Leydig cells and Sertoli cells, an evidence quite convincing according to Means (1976). Testosterone of Leydig cell origin can be aromatised into oestrogen only in 20-day old rats, the T forming a substrate for oestrogen synthesis (Armstrong & Dorrington 1977). Even cholera toxin can bring about the stimulation of aromatization (Setchell 1978). Of the two androgens in the testis, T and DHT, only the former can be aromatised (Davidson *et al.* 1977). Adult culture of Sertoli cells was found to form exogenous progesterone (Tchalokian & Steinberger 1978). This messenger system, was, speculated as nothing but an internal negative feedback loop between the Sertoli and Leydig cells monitoring androgen via oestrogens (Steinberger *et al.* 1979). Oestrogen formation in the testis according to Wilson (1975) 'involves sequentially the hydroxylation, oxidation and removal of the carbon at the 19 position of the steroid molecule and aromatization of the A ring of the steroid'. While the aromatization of T into oestradiol in the Sertoli cells is generally accepted, Steinberger *et al.* (1979) brought out that the Sertoli cells do metabolize T into a steroid which has immuno-characteristics and chromatographic behaviour like oestradiol-17β but its identity is not proven; it must be proved that the oestrogens (Oe_1, Oe_2) recrystallise to constant specific activity (see also Tchalokian & Steinberger 1979). Dorrington *et al.* (1978a) stated that after incubating Sertoli cells from 20-day-old rats with deuterated T, deuterium lebel appeared in the isolated oestradiol.

The residual bodies (rich in enzymes derived from spermatids) undergo degeneration by autolysis (disappearance of RNA and basic protein) (Vaughn 1966). According to Austin (1972) when the residual body is sloughed, the 'meiotic' RNA goes out with it.

7c *Ageing and Sertoli Cell Behaviour*

In man, as age advances, the Sertoli cell becomes more prominent since spermatogenesis declines (Leathem 1977); Harbitz (1973) also noted such a decline.

During examination of more than 141 cases, the Sertoli cells stained with phosphotungstic acid-haematoxylin appeared to acquire ascorbic acid and glycogen as age advances. They also showed greater lipid accumulation,—starting at 15 years of age and increasing in amount till 84 years (Lynch & Scott 1950).

7d *Nucleus of Sertoli Cells*

The nucleus is infolded or lobulated with perforated nuclear envelope (figure 16); the fibrous lamina on the inner face of the nuclear envelope in many cells is lacking in the Sertoli cell. The DNA content of the Sertoli cell (in the mouse) is tetraploid or octaploid (Swift 1950) and remains unchanged (Daoust & Clermont 1955).

The Sertoli cell and its nucleus may undergo reduction in size after the release of the spermatozoa (Regaud 1901).

The nucleolar complex: The nucleolus itself is situated in the centre of the nucleus flanked by justanuclear bodies which are nucleolus-associated heterochromatin. EM studies (Fawcett 1975) have indicated that the central body has the texture of a nucleolonema (Feulgen-negative) and the two lateral bodies that of a condensed chromatin (also called perinucleolar spheres or satellite karyosomes or heteropycnotic bodies) and are also Feulgen-negative.

7e *The Sertoli Cell-Sertoli Cell Tight Junctions* (figures 16, 18, 19)

It has already been noted that the Sertoli cells act as supporting cells and also protect the germ cells. They prevent any matter in blood as e.g., environmental toxic mutagens (Neaves 1977a) from coming in contact with the spermatocytes, spermatids and sperms by forming a blood-testis barrier (Chiquoine 1964), or it can also be called seminiferous-tubule barrier. This barrier is formed, as revealed by EM between pairs of adjacent Sertoli cells coming together (above the spermatogonia and below the spermatocytes) at this region forming tight linear junctions (electron lucent) alternating with areas where intercellular space is noticed appearing electron-dense in lanthanum-marker-filled preparations (Kumamoto & Furaya 1976). Generally, in mammals including man (Kumamoto & Furaya 1976), on either side of the tight junctions (zona occludens) of the Sertoli cells, the endoplasmic reticulum becomes modified into subsurface cisternae; these cisternae show the presence of ribosomes on the side away from the cell membrane (figure 18). Further, there are bundles of microfilaments in the region of the tight junctions and also beyond, which in a cross-section present a stippled appearance. These filaments have been found to be actin-like (Tomaya 1975) and it has been postulated by Gilula *et al.* (1976) that the contractility of these actin fibres disposed like a ring round the base of the Sertoli cells causes a clone of germ cells to move up. Fawcett *et al.* (1976) also postulated that adjacent Sertoli cells may send processes which meet below the luminal movement of germ cells; where the Sertoli cell processes meet, a tight juncion is formed to maintain the integrity of a blood-testis barrier while the junction above the basal compartment disorganizes or an unzipping take place. A specific protease (plasminogen activator) may help in spermiation or opening the occluding junctions for the movement of germ cells (Lacroix *et al.* 1977). Ross and Dobler (1975) made an interesting suggestion with regard to germ cell movement. They postulated the separation of tight junctions and when the spermatocytes have moved up, the

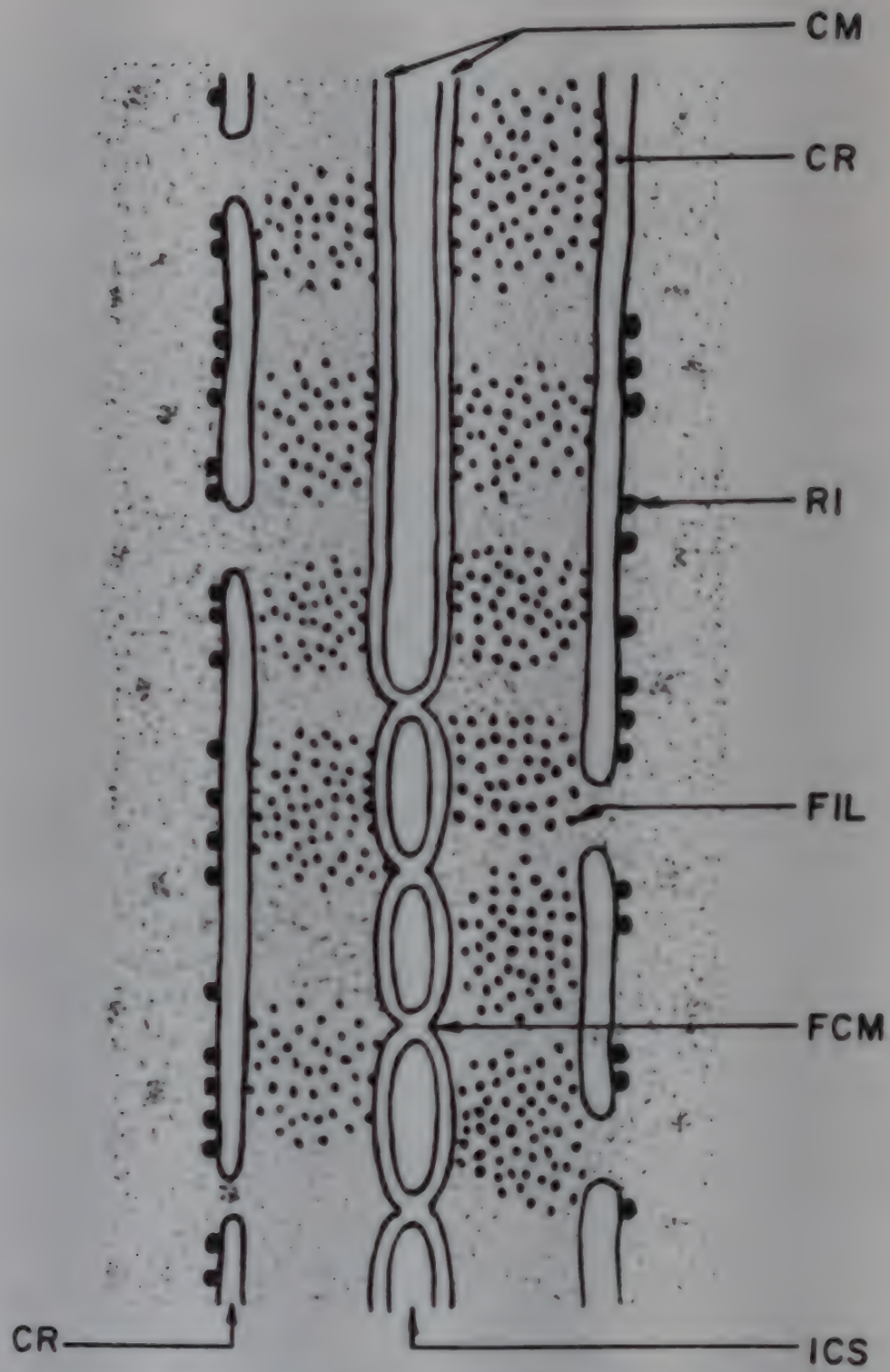


Figure 18. Diagrammatic representation of a part of blood-testis barrier, from ultrastructural studies. The double layered cell memberane of two adjacent Sertoli cells have fused in certain places forming tight junctions. Actin filaments are seen as dots The endoplasmic reticulum has formed cisternae showing ribosomes on the side away from the cell membrane (after Fawcett 1975, modified)

hemi-junction would adhere to the germ cell higher up and this contact between spermatid progeny and the inter-Sertoli cell junction is maintained till the spermatids are released into the tubule. Unfortunately, no hemi-junctions are noticed in sections; according to Nagano and Suzuki (1976), no junction between the

spermatogonium and the Sertoli cell is present. Burgos *et al.* (1970) brought out half Sertoli cell junction at the surface of spermatocytes, early and late spermatids and spermatozoa.

Referring to Fawcett's figure (figure 16), Neaves (1977a, b) described that the basal zone contained the spermatogonia and preleptotene spermatocytes while the apical zone had advanced spermatocytes and spermatids. While Dym and Fawcett (1970) consider that the basal compartment has the spermatogonia and preleptotene spermatocytes, and the adluminal the more advanced spermatocytes and spermatids, Fawcett *et al.* (1976) stated that the basal compartment had the stem cells of spermatogenesis. Dym and Cavicchia (1977) reported that the basal compartment contained A and B spermatogonia, preleptotene and leptotene spermatocytes, and the adluminal compartment the zygotene, pachytene, diplotene and secondary spermatocytes and spermatids. The Steinbergers (1977a) stated that the basal compartment had the spermatogonia and the rest were in the adluminal compartment (see also Bloom & Fawcett 1976; p. 841).

A blood-testis barrier does not occur in immature testis; its appearance seems to be coeval with that of spermatogenesis and of tubular lumen for the gametes (Neaves 1977a,b). Not only that; at the time of differentiation of Sertoli cells, the latter's response to FSH is dramatically reduced coinciding with the formation of tight-junctions between Sertoli cells. The tight-junctions are formed at about 20 days of the postnatal life of rat (Gilula *et al.* 1976, Means *et al.* 1980), when also increased cAMP, inhibition of phosphodiesterase, activation of protein kinase, RNA and protein synthesis and mitotic activity all seem to reach a surge. The junctions besides various other functions, allow to pass selectively some germ cells (spermatocytes, spermatids) but not the spermatogonia when spermatogenesis starts. That the tight junctions are manifesting themselves is heralded by the appearance of microfilament bundles juxtaposed to the regions of future fusion of the cell membranes (Connell 1978, Gilula *et al.* 1976). In addition, smooth endoplasmic reticulum (SER) is also found associated with junctional areas. When the tight junctions separating the basal (containing only spermatogonia) from the adluminal (containing the other division products) compartments disorganize or unzipper to allow the spermatocytes and spermatids to move into the adluminal compartment, the half junctional complexes with the characteristic thickened Sertoli plasma membrane, the SER and the microfilaments are still there. When a new membrane is synthesized and the Sertoli cytoplasmic processes enlarge, the half junctional complexes are found attached to the spermatid heads during spermatogenesis (Russell 1977b). The movement of the germ cells from the basal to the adluminal compartments appears to be controlled by the microfilament and SER activity. Means *et al.* (1980) discuss that in expelling the spermatids, the SER acts as a calcium store-house and that the cAMP regulates the passage of calcium from a bound to a free state; thus freed calcium may get associated with calmodulin. The latter is a highly constitutive protein and is the intracellular receptor for calcium whose intracellular concentration is unaffected by many a peptide hormones. The calcium having got associated with calmodulin causes generation of motile force of the microfilaments. Very likely this force allows the expulsion of the developed spermatids through a microfilament contraction-relaxation cycle.

It has also been brought out by Tindall *et al.* (1975) that in 14-day-old rats (15–18 days postnatally according to Steinberger & Steinberger 1977a), androgen-binding protein is found in the testis but not in the epididymis. Similarly in irradiated mother rats, the male offspring show the 'Sertoli cell only' testis with no germinal cells. In these Sertoli cells at 30 days, the tight junctions, tubular lumen and polarised secretion of tubular fluid (Purvis & Hansson 1981) appear and the ABP finds its way into the epididymis. Busulfan injection to pregnant mothers also causes the 'Sertoli-cell only' testis and these offspring develop the characteristic blood-testis barrier indicating that the absence of seminal cells does not prevent the formation of the barrier (Means 1974, Neaves 1977a, b). In the rat and guinea pig, it has been described that in addition to the Sertoli cell-Sertoli cell junction, the myoid cells (= peritubular cells of Hansson *et al.* 1976) in the tubule wall (tunica propria) form tight junctions stopping markers like peroxidase and lanthanum from entering further. In man and monkeys, the formation of a myoid cell tight junction is not noticed (Dym 1973, Setchell & Waites 1975).

The blood-testis barrier appears to be less rigid in permitting entry of substances into seminiferous epithelium at the region of the tubulus rectus. Generally, the blood-testis barrier does not allow lymphocytes to pass through it and enter the seminiferous epithelium. But in the terminal regions of the tubule, there are fewer Sertoli cell junctions and these obviously permit the escape of lymphocytes as these are found in the seminal epithelium here (Dym 1974).

Setchell and Waites (1970) describe that cadmium salts cause greater permeability of the blood-testis barrier; it is also known that damage caused by autoimmune reaction breaks the blood-testis barrier. The number of Sertoli cell junctions appears to increase in animals with testosterone implants (Bressler 1978).

The possible functions of the blood-testis barrier may now be recounted (Neaves 1977a,b) :

(i) to prevent deleterious substances circulating in the blood [as e.g., environmental toxins and mutagens (Neaves 1977a,b) as already said] from coming in contact with the germ cells. This barrier appears to be discriminatory (Purvis *et al.* 1977). Tracer molecules like ferritin, horseradish peroxidase, certain blood proteins like albumin and cholesterol do not pass through. Hormones like FSH and LH, certain sugars, amino acids, carbohydrates and steroid hormones can pass into the tubular part. Androgens appear to have preference in passing into the adluminal compartment.

(ii) the barrier prevents autoimmune orchitis (or allergic lesion of the testis), a function very necessary for the survival of the species. There are two facets of protection against this allergy: (a) the sperm-specific auto-antigens should be isolated in the seminiferous tubules, and (b) to exclude the sperm-specific auto-antibodies and autoimmunocytes from the tubule if by any chance sensitization does occur (Neaves 1977a,b).

(iii) it enables the epithelium to secrete by making the Sertoli cells to pump solute into the clefts in the barrier so that the osmotic gradient created would move fluid from the base to the lumen.

(iv) the barrier helps the Sertoli cells to create the necessary milieu in the adluminal segment where germ cells may differentiate. Dym (1974) thought that Sertoli cells provided a special environment for meiosis and spermatogenesis to proceed in the testis tubule. When the preleptotene spermatocytes moved from the basal to adluminal zone, this committed them to enter meiosis (Setchell & Waites 1975; Setchell 1978). It has now been found that in the monkeys (Dym & Cavicchia 1977), the late leptotene or early zygotene spermatocyte cell type migrates from the basal to the adluminal compartment at stages IX-X of the cycle. This means that meiosis starts even when early spermatocytes (preleptotene and leptotene) are still in the basal compartment thus disproving the necessity of a special milieu created by the Sertoli cells for initiating meiosis. In other words, initiation of meiosis can take place in a milieu similar to other cells of body but progress of meiosis and further maturation of germ cells need a specific intratubular milieu.

(v) Setchell (1978) refers to a very important function of the barrier (Suominen & Setchell 1976). This barrier helps in maintaining a high concentration of a peptide which prevents the acrosomal proteinase or 'acrosin' to penetrate the ovum. Acrosin can allow spermatozoa to penetrate any cell and therefore, the inhibitory peptide is present at a time when the sperm leave the germinal epithelium.

(vi) It may be that the blood-testis barrier is providing favourable conditions for the synthesis of nucleic acids in the seminal tubules, as bases like glutamic acid, aspartic acid and alanine are synthesized and their high concentration maintained there (Waites & Setchell 1969).

In studying the biogenic amines, Urry and Ellis (1977) brought out that mono-amine oxidase may act as a part of the blood-testis barrier in preventing the entry of biogenic amines into the seminiferous tubules.

While the above conclusions are generally accepted, Aragon and Lustig (1973) stated that the uptake of horse-radish peroxidase was not only beyond the tight junctions but also in the central lumen. According to them, the so-called blood-testis barrier was only partially effective and that it was discontinuous along the length of the tubule. This challenge was met by repeating the experiment more rigorously by taking all necessary precautions, and Aoki and Fawcett (1975) came to the conclusion that what Aragon and Lustig (1973) had reported may be due to technical hazards of their methods.

The Fawcett school brought out the absence of typical desmosomes in the seminiferous epithelium and the absence of any cell attachments in the upper two-thirds of the epithelium to facilitate upward movement of germ cells and this was challenged by Ross (1976). According to him, the Sertoli cell junctions are permanent features through an entire seminiferous epithelial cycle. The junctions help in the attachment of Sertoli cells forming occluding ones and later, the same junctions serve for the attachment of spermatids. The filaments and cisternae do detach and allow the movement of spermatocyte into the adluminal compartment or for the release of sperm. Further, it is the acrosome of the spermatid which causes the area of adhesion to the Sertoli junctional specializations. This Sertoli-

spermatid attachment is present throughout meiosis and germ cell development. The loss of adhesiveness of the Sertoli cell would cause the release of sperm.

Dym and Cavicchia (1977) described that in the monkey (figure 19) some zygotene and many pachytene spermatocytes were bonded with half a Sertoli-Sertoli junction, supporting the observation of Ross (1976). Such hemi-junctions were few around round spermatids and were associated with the spermatid's acrosome during the elongation phase. It is likely that the original Sertoli-Sertoli cell junctions persist during germ cell activity; their reduction during early spermiogenesis and reappearance when the round spermatids elongate leads one to conjecture that they dissolve and then are reformed.

7f *Movement of Germ Cells from Basal to Adluminal Compartment*

The diagrammatic figure (19) reproduced (Dym & Cavicchia 1977) shows (top row) that the spermatogonia are sitting right on the basal lamina and the tracer compound enters only between them and the Sertoli cell but not beyond as there are tight junctions. When these cells have to move up, these tight junctions have to break down and new ones formed beneath so that the integrity of this arrangement is not lost. The above authors note that the new junctions beneath the preleptotene and leptotene cells reform (stages VII-IX of the cycle) before the tight junctions disorganize (or unzipper) above the zygotene and early pachytene cells (stages X-XII-I of the cycle). Thus there appear two barriers, one above and the other below,—a transient 'intermediate' compartment (figure 19, middle row) as reported by Russel (1977a). But tracer studies have not supported the formation of an intermediate compartment as lanthanum and peroxidase markers do encircle preleptotene, leptotene spermatocytes and spermatogonia and spread from one to other germ cell as they are syncytially connected. Many of the zygotene spermatocytes have pre-existing tight junctions above and many of them appear split into two halves and each half may reposition by the side of the said spermatocyte (figure 19, bottom row).

Fawcett *et al.* (1976) pointed out that the attachment of the spermatocyte or spermatid with one-half of the Sertoli cell junction has rarely been observed. At the Sertoli cell junctions there is a firm fusion of cell membranes at the tight junctions caused by the linear fusions of the opposing membranes. In studying this, the freeze fracture technique has helped considerably. It is noticed that there are intramembranous anastomosing rods or rows of particles underlying the linear attachments. At the Sertoli-spermatid junctions, lanthanum penetrates, and therefore, there can be no fusion as Ross would have it. Obviously, more work is called for in this field.

That there is a communication between adjacent Sertoli cells and between the Sertoli cell and germ cell, is gaining ground. Between the Sertoli cells, it involves a 'flux of ions and small molecules' through the gap junctions. Between the germ cells and the Sertoli cells, there are no junctions but all the same exchange may be possible. The aggregation of spermatid mitochondria at the cell surface and that of smooth endoplasmic reticulum adjacent to the heads of maturing spermatids (figure 16) is suggestive of it (Fawcett 1975).

The blood-testis barrier resists hypertonicity (Nagano & Suzuki 1976);

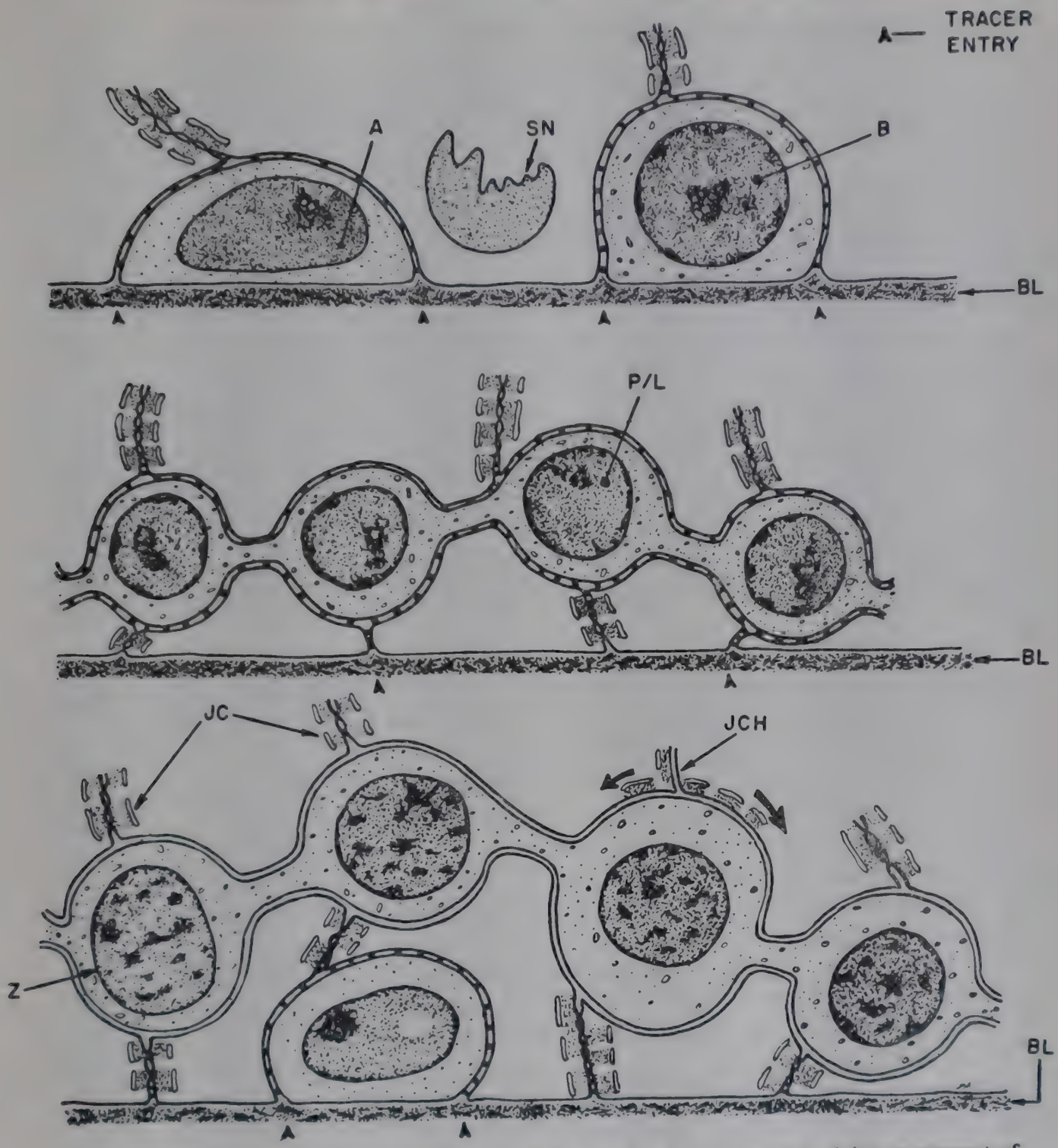


Figure 19. Diagrammatic representation of the fate of Sertoli cell tight junction duing movement of germ cells from basal to adluminal compartment. In the top one, tracer has entered all around types A and B spermatogonia. Arrows indicate the place of entry of the tracer. In the middle set of preleptotene and leptotene spermatocytes, a new set of tight junctions is formed above while the ones nearer the basal lamina are breaking down. In the lowest set, the tight junction separates into two halves (large arrows). Each half re-positions by the side of the zygote spermatocytes (after Dym & Cavicchia 1977, modified)

hypertonic solution (of lithium chloride, urea or sugar) does not affect the adluminal compartment while the cytoplasm of the spermatogonia of the basal compartment undergoes great shrinkage (appearing empty) and also some condensation of Sertoli cell bases (Nagano & Suzuki 1976, Gilula *et al.* 1976).

8 BIOCHEMISTRY OF THE SERTOLI CELL

The cytoplasm of the Sertoli cell ramifies among the spermatogenic cells and it has already been noticed that the spermatids are intimately associated with the Sertoli cells free end. The ingestion of residual bodies may be a source of lipids which are released into the tubule lumen—a source of nutrient for the sperm in the lumen (Free 1970). Fawcett (1972) suggested that the smooth endoplasmic reticulum closely associated with the acrosome cap may secrete some lipid or steroid factor which may shape the acrosome cap. Vilar *et al.* (1962) also pointed out that the branches of the Sertoli cell take the place of intercellular spaces and food from the intertubular blood vessels may have to pass through the basal membrane and the Sertoli cell itself to reach the germinal cells. Glycogen is often found in the Sertoli cell and shows a variation; this is taken to mean that during spermatogenesis, the spermatids utilize this substrate. Nicander (1957) also brought out that glycogen was found round the heads of spermatids during spermatogenesis and towards the end of the cycle, it diminished. In the stallion and cats, on the other hand, it increased at the end of the cycle. In rat, mouse and dog no glycogen was seen in the Sertoli cell. Firlit and Davis (1965) opined that the Sertoli cell may synthesize glycogen from blood glucose or from the phagocytosed residual body in the Sertoli cells.

In the two types of cells in the tubule, viz., the Sertoli cells and the spermatogonia, maximum pentose cycle activity is noticed (Free 1970). As nucleic acid synthesis goes on in spermatogonia, a demand for ribose is answered by pentose cycle activity.

In addition to glycogen, a certain amount of lipid may also be found in the Sertoli cell. Johnson (1970) stated that the residual body left by the extruding sperm in the Sertoli cell is composed, in part, of lipid; as is known, a part of the residual body enters the testis tubule lumen. In the human testis, the Sertoli cell showed a gradual increase of lipid with age from 11 onwards (Lynch & Scott 1950).

With regard to nucleic acids of the Sertoli cells, Gledhill (1970) stated that using the Feulgen technique, the DNA distribution in the cell remained constant during all the stages of the cycle. Using DNase and staining with pyronin, an intense reaction was seen in the nucleolus. Further, using ^{3}H-Uridine as a specific precursor of RNA, it was noticed that the nucleoplasm and nucleolus incorporated it. However, Monesi (1965) feels that both nucleus and nucleolus synthesize RNA, as both nucleoplasm and nucleolus of Sertoli cell incorporate ^{3}H-Uridine (a specific precursor of RNA) but not the satellite karyosomes.

8a *FSH and the Sertoli Cell*

Students of male mammalian reproduction may be familiar with the action of gonadotropins during spermatogenesis. FSH acts on the Sertoli cells of the testis tubules and LH acts on the intertubular Leydig (interstitial) cells mainly. Considerable work has been done on the biochemical changes in the Sertoli cells when FSH acts *in vivo* and *in vitro*. Means has reviewed the work done by his School and by others (Means 1975, 1976, 1977, Means & Hall 1967, Means & Huckins 1974, Means & Tindall 1975, Means *et al.* 1976, 1978a, 1978b). Steinberger *et al.* (1974) have also reviewed the subject critically.

The Sertoli cell is the primary target for FSH. This was suggested as early as 1965 by Murphy. Means *et al.* (1980) recorded at least eight biochemical activities monitored by the gonadotropin; they are: (a) ion flux, (b) enzyme activity, (c) protein synthesis, (d) protein secretion, (e) steroid synthesis, (f) cell division, (g) cell motility, and (h) cell-cell communication.

FSH appears to recognize and bind itself to specific receptors on the plasma membrane of the Sertoli cell. These receptors are partly protein and have a phospholipid content also on the membrane essential for its function (Dufau *et al.* 1973). It is known that immature rats respond to single doses of FSH better than mature ones and the maximum concentration of the receptors is achieved by 5–16 days postnatally in the rat and the maximum effect of a single dose of hormone is noticed in 10-day-old rats. The peptide hormone activates within 1–2 hr of injection membrane reaction and sets up a series of biochemical changes. The plasma membrane-bound adenylate (adenylyl) cyclase (figure 20) is activated; intracellular concentration and metabolism of a cyclic nucleotide (cAMP = cyclic adenosine 3^1-5^1-monophosphate) increases. *Pari passu* the activity of the calcium-dependent cAMP-phosphodiesterase is lowered (Means 1976). The concentration of cAMP promotes dissociation of inactive cAMP-dependent protein kinase (PK) and free the catalytic subunit of this enzyme (Means *et al.* 1980).

This latter reaction is dependent on time, temperature of reaction and concentration of the gonadotropin. The gonadotropin can activate PK in 16-day-old rats but not in 30-day-old ones and, therefore, age also seems to play an important role. In the tubules, 16-day-old rats showed pachytene primary spermatocytes; the biochemical changes disappear between 21–24/26 days of age of rats when the testis does not react to exogenous FSH. This is also the time when active spermatogenesis is noticed in the testis. In mature rats, the receptor sites again manifest themselves in the Sertoli cells. The loss referred to above may be due to the nucleotide accumulation, protein kinase activity and later protein phosphorylation.

The catalytic subunit of PK phosphorylates proteins present in every subcellular chamber of the Sertoli cell. Though the tempo of RNA and protein synthesis is elevated, no direct correlation with altered cyclic nucleotide metabolism could be established (Means *et al.* 1976).

There are four proteins in the Sertoli cell whose levels are definitely stimulated by FSH: (a) ABP, (b) plasminogen activator (Lacroix *et al.* 1977), (c) protein kinase inhibitor (Beale *et al.* 1977), and (d) gamma glutamyl transpeptidase (Lu & Steinberger 1977).

There is one other enzyme,—phosphodiesterase (PDE) (figure 20) playing a big role in the biochemistry of the Sertoli cell. A major isozyme of PDE, it is capable of hydrolysing or degrading cAMP to 5'AMP. Probably the latter reacts with the receptor-adenylate cyclase system and potentiates FSH towards production of cAMP. 5'AMP has been described to interact directly with adenylate cyclase. Treatment of immature testis with FSH inhibited PDE while this did not happen in the adult. What is the possible cause of this inhibition? Means has quoted reports of the presence of a heat-stable Ca^{++}-binding protein activating the continued functioning of adenylate cyclase. In immature Sertoli cells, changes in calcium concentration

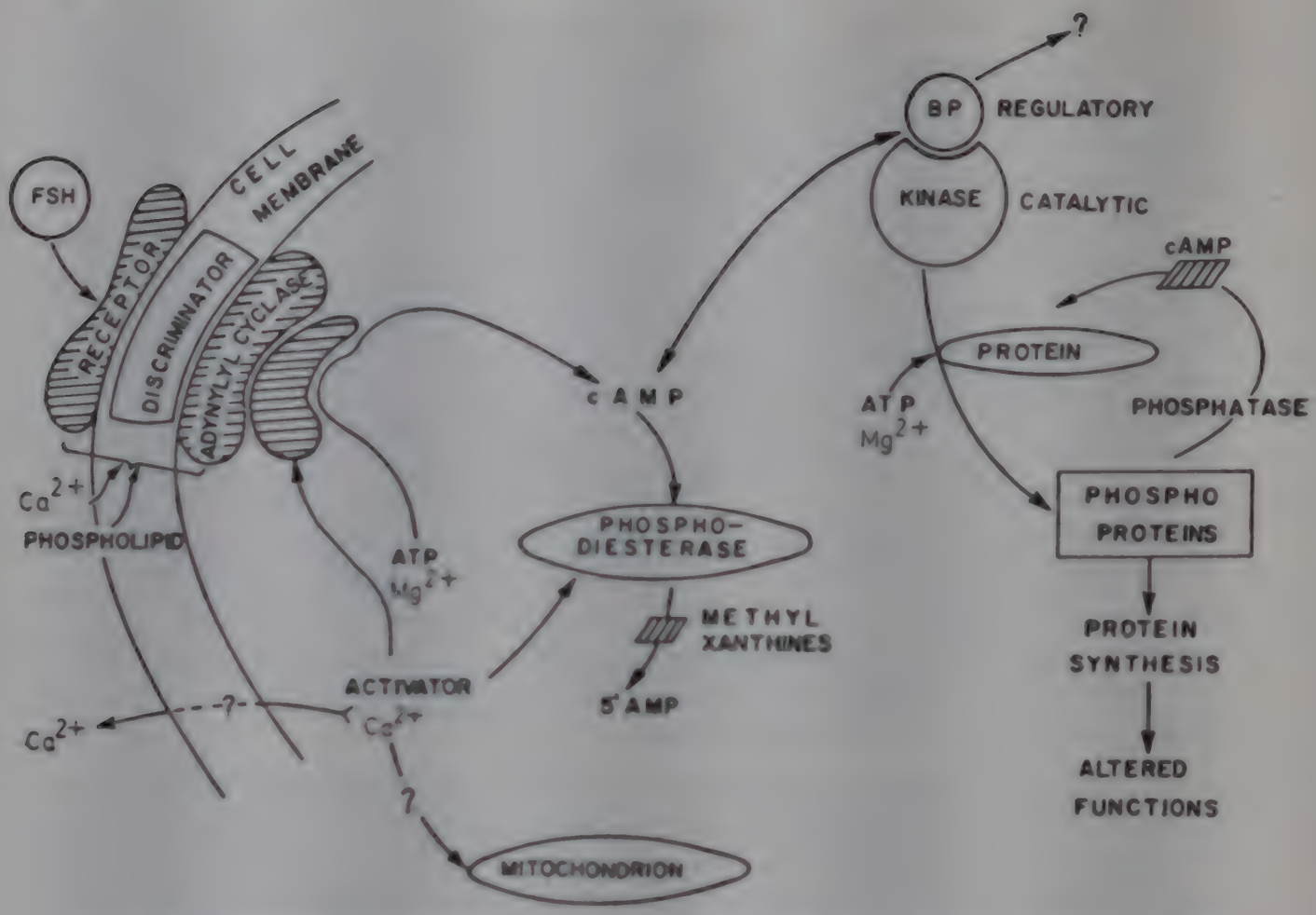


Figure 20. Schematic representation of some early events in the action of FSH on Sertoli cell membrane, of cyclic nucleotide synthesis and metabolism (after Means 1976, modified)

would alter PDE activity which does not happen in the adult. The Means School has described the occurrence of binding protein in the Sertoli celland goes to show that FSH affects the transport of Ca^{++}.

It was pointed out above that FSH does not activate PK in 30-day-old rats. Probably the gonadotropin actions are governed by PDE. If an inhibitor like 1-methyl,3-isobutylxanthine (MIX) of the enzyme PDE is used, the PK activity goes up as also the intracellular levels of cAMP. One could infer that in mature rats, PDE is playing a part in modulating or regulating FSH action. What has been enumerated above has been put schematically by Means (1975, 1976, 1977), and I have reproduced the same (figure 20). A similar schematic representation of the hypothetical scheme of the action of hormones (no mention is made of any specific hormone) has been given by Hoskins and Casillas (1975). Their hypothesis is based on the work of Sutherland (1972) and of Krebs (1972).

FSH synthesizes in the Sertoli cell a protein which influences both PK and PDE. It is a low-molecular-weight, heat-stable molecule which is reported to inhibit the catalytic part of the cAMP-dependent PK and the calcium-dependent cAMP-PDE (Beale *et al.* 1977), and is called protein kinase inhibitor.

Adenylate cyclase is the only enzyme known at present to synthesize cAMP and the cyclic nucleotide-dependent PDE is the only enzyme to degrade cAMP

(Means *et al.* 1980). Means *et al.* (1979) have represented this in the form of a model, which I have reproduced with minor modifications:

ATP → adenylate cyclase → synthesis of cAMP → degraded by PDE → 5'AMP

This kinetic system is found to be irreversible.

The cAMP-PDE exists in two isoforms in tissues; only one of them is calcium-sensitive. When physico-chemical properties of these two were studied, it was noticed that, (a) the calcium-dependent enzyme hydrolyses both cyclic guanosine monophosphate (cGMP) and cAMP (form 1), and (b) the other does not need calcium and only utilises cAMP at physiological concentrations (form 2). The distribution of enzymes is different in immature and mature forms. In the latter, there is a third isoform of PDE which utilises cGMP and heat-stable calcium-dependent regulator protein (CDR). Form 1 PDE described above, is absent from mature Sertoli cells. Steinberger *et al.* (1978) have it that assumption of a second form of PDE remains to be proven. Setchell (1978) also brought out the same point from ion-exchange chromatography in the testes. One is calcium-dependent and hydrolyses both cAMP and cGMP and is present in immature animals; the second is also calcium-dependent and hydrolyses only cGMP and is present only in mature animals; and the third isozyme is not calcium-dependent and is specific for cAMP. Purvis and Hansson (1981) also feel that there are multiple PDEs with different substrate specificities and different kinetic behaviour.

8b *In vitro Culture of Sertoli Cells*

Means *et al.* (1979) also studied the shape of monolayer culture of cells under the influence of FSH. When FSH and cAMP or EGTA [= ethylene dioxybis (ethylenamino) tetraacetic acid, a calcium^{2+}-chelating reagent] is added, the cells become rounded which could be reversed by adding calcium. Even Sertoli cells from mature animals reacted in the same way. Neither LH nor testosterone caused this change. Porter (1966) reported that the cell shape depended on the integrity of the cytoplasmic microtubule complex.

In studying the cytoskeletal architecture under the influence of calcium or FSH, the indirect immunofluorescence technique was helpful. The Sertoli cell disclosed that from a highly fluorescent centre associated with centrioles, there are radiating microtubules. It appears that the microtubules do not control the shape of the Sertoli cell. Means *et al.* (1979) then tackled the microfilaments. These showed the presence of two protein components, actin and myosin, like the contractile muscle fibre. Using a microfilament breaker, cytochalasin B, it was found that all actin fibres (stress fibres) disappeared. As a result, the Sertoli cell changed its shape. One could infer that the interphase Sertoli cell shape is determined by the microfilament system. If FSH is added or calcium removed from the Sertoli cell, the microfilament network is disarranged leading to a change in shape.

It is noticed that FSH brings about in the Sertoli cell on the one hand, a fall in PDE activity, and on the other, an increase in non-exchangeable calcium, both working through calcium. In trying to reconcile the above, one should also take into account the effects due to FSH and the decreased calcium levels with regard to the microfilaments and cell shape. The calcium dependent regulator (CDR) to which reference was made plays a key role. Gonadotropins (FSH, LH) and testosterone

do not bring about any significant differences in the level of CDR (Tash *et al.* 1979). A reference was made to a protein kinase inhibitor (PKI) in the Sertoli cell. This protein is associated with microtubules and FSH causes a significant increase of it (Tash *et al.* 1979). Briefly CDR is an acidic thermostable protein which binds calcium at physiological concentrations in all tissues.

In resume, there appear to be three processes in the Sertoli cell affected by Ca^{2+} and FSH. These are, (a) regulation of Ca^{2+}-dependent PDE, (b) regulation of intracellular Ca^{+} level, and (c) control of microfilaments. CDR apparently plays a part in the third one.

FSH has probably no regulatory effect on the total CDR concentration in the Sertoli cell (Means *et al.* 1979). Calcium appears to be monitored by FSH such that PDE and CDR are regulated independently. It is likely that FSH through Ca^{2+} and CDR controls movement of germ cells from the basal to adluminal compartments. Probably the tight junctions with their microfilaments may also be controlled by CDR-Ca^{2+} system. Sertoli cell secretes proteins and other organic molecules into the tubule fluid and these appear to be influenced by Ca^{2+}. Microfilaments containing actin (myosin?) and the microtubules appear to be involved in secretion; the former probably provide the motile force for granule movements, and the contraction-relaxation being under the calcium regulation (Dedman *et al.* 1979), and the latter, i.e. the microtubule, is the framework to define and orient the flow of secretory granules (Means *et al.* 1980). Based on the work done so far with regard to biochemical events consequent upon FSH action on Sertoli cell, the Means School (Means *et al.* 1980) put forward a model with regard to FSH and exocytotic secretion of substances synthesized in the Sertoli cell. It was pointed out that the active catalytic subunit of the protein kinase phosphorylates proteins in every subcellular compartment in the Sertoli cell. One such is the substrate which is phosphorylated in the myosin light chain kinase (Adelstein *et al.* 1978) which is abundantly present in the Sertoli cell. This myosin light-chain kinase (cyclic nucleotide-independent protein kinase) upon activation combines with calmodulin and promotes phosphorylation of the light chain of myosin. Calmodulin is present in all eukaryotic cells and is a calcium-binding protein (Dedman *et al.* 1979); it is present in the cell associated with the plasma membrane and also its cytoskeleton, viz., microtubules and microfilaments (Dedman *et al.* 1979). Now this results in the combination of actin with myosin in heavy chain and this stimulates ATPase (Scardolis & Adelstein 1977). Hydrolysis of ATP releases energy for restructuring microfilaments. The events enumerated above have been tested further. A graphic representation of temporal sequence of events in Sertoli cells which secrete proteins by exocytosis is envisaged by Means *et al.* (1980) and Welsh *et al.* (1979, in press) (figure 21).

8c *The Bonding of FSH*

Till recently, the idea was that FSH bound itself to the receptor at the plasma membrane of the Sertoli cell and exerted its influence on cell physiology. In 1978, Petrusz (1978) put forward the view (figure 22) that the hormone is carried internally into the cytoplasm (see LH receptors and Leydig cell). The hormone is bound to the receptor present on the plasma membrane and this complex is taken into the cell by vesicular-uptake (pinocytosis, endocytosis or encytosis). The endocytotic vesicles

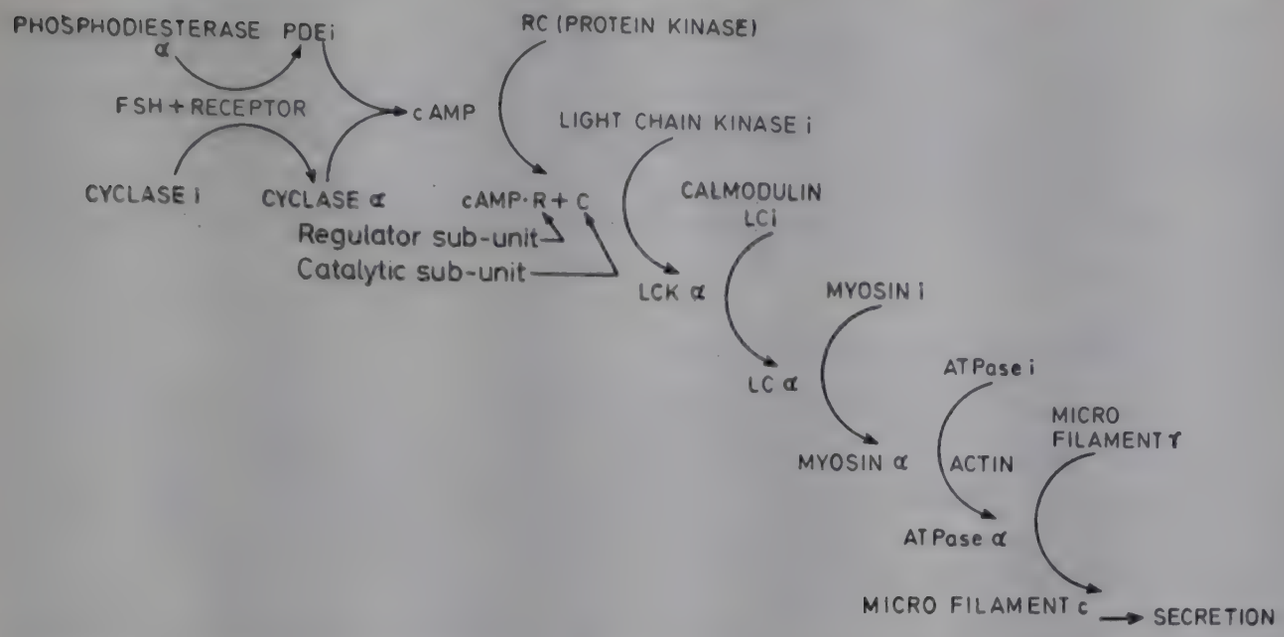


Figure 21. Diagrammatic representation of the temporal sequence of events in Sertoli cell which secretes protein by exocytosis (after Means *et al.* 1980, modified)

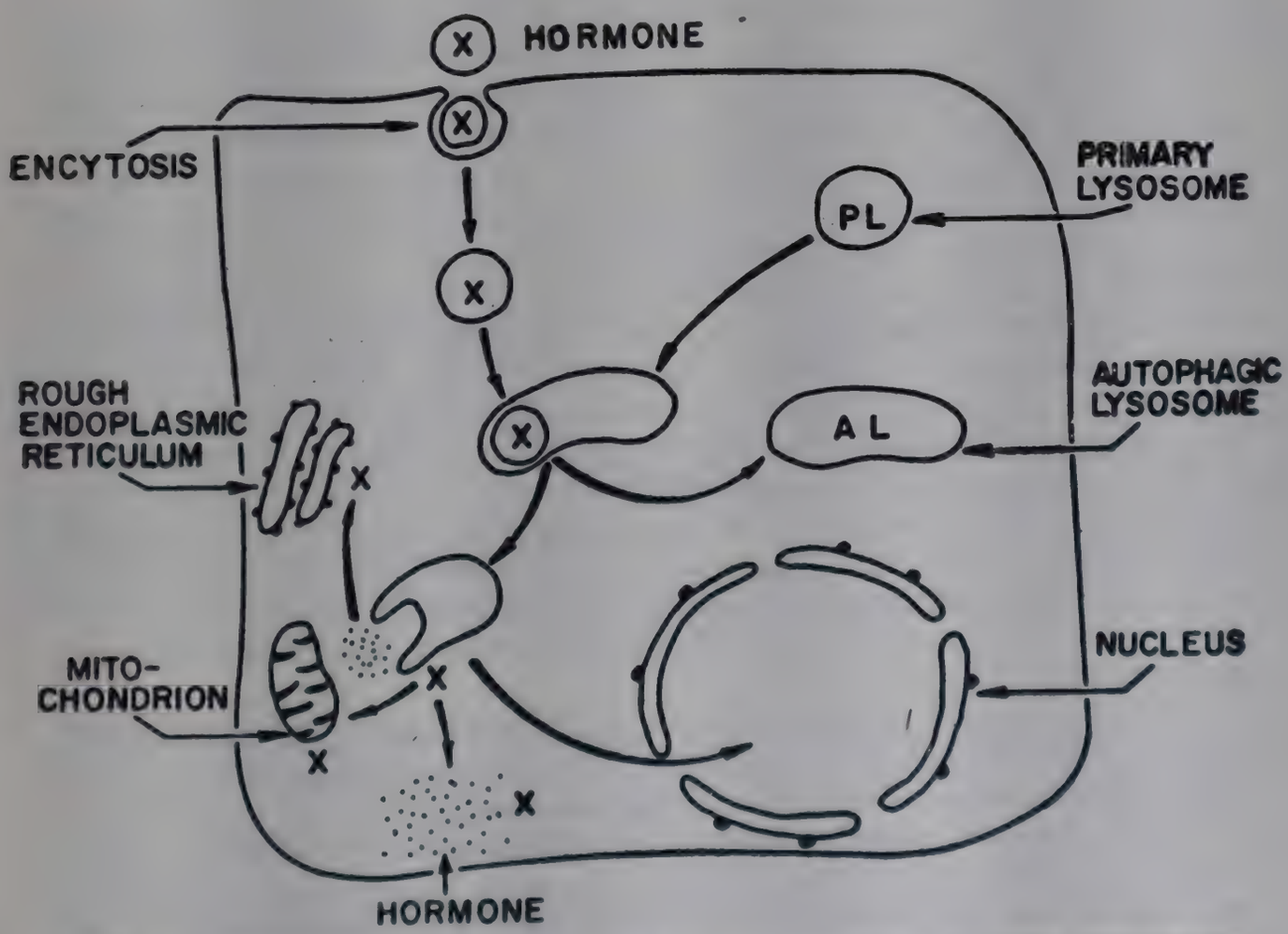


Figure 22. Diagrammatic representation of how gonadotropic hormones enter cells and their subsequent fate (after Petrusz 1978, modified)

then fuse with primary lysosomes to form secondary lysosomes. The hormone and probably the receptors are inactivated by lysosomal enzymes as in the autophagic lysosome. Or a regulatory lysosome becomes de-stabilised and the hormone escapes into the cytoplasm; the hormone may get into subcellular organelles or get into the nucleus through its pores. Or the hormone may become broken down and these particles may pass through the lysosomal membrane. Cellular regulatory processes ensue.

8d *The Androgen-binding Protein (ABP)*

The Means School (Tindall *et al.* 1974) brought out direct evidence that ABP is synthesized in the Sertoli cell while French *et al.* (1974) adduced indirect evidence of its synthesis. Sanborn *et al.* (1976) have hypothesized that the gonadotropin FSH initiates a series of activities in the Sertoli cell. The precise mode of synthesis of ABP is unclear; it is likely that both FSH and testosterone control it (Louis & Fritz 1979); Hansson *et al.* (1977) opine that T is the primary stimulus for intracellular synthesis, probably by two tropic hormones, FSH and androgens (Purvis & Hansson 1981). The secretion of ABP is specifically controlled by FSH (Hansson *et al.* 1977, Means *et al.* 1979). It is also reported that mitotic activity of immature Sertoli cells increased due to FSH (Solari & Fritz 1978).

FSH is bound on the plasma membrane of the Sertoli cell (figures 23, 24) to a specific membrane receptor (absent, however, from interstitial cells, peritubular myoid or germ cells, Steinberger *et al.* 1974) and activates adenylate cyclase which in turn activates cAMP. The latter causes the synthesis of proteins from the DNA-dependent RNA; one of these proteins is ABP. The ABP moves into intercellular spaces in the tubule wall where T has diffused from Leydig cells by passing through the same tubule wall. Nieschlag and Wickings (1977) also feel that T elaborated in the Leydig cells reaches the tubule directly and not via the blood circulation. Androgen is bound to the ABP. In the intercellular spaces, T cleaves itself from ABP and gets bound to the cytoplasmic androgen receptor on the germ cell wall (figure 25). This receptor complex translocates into the nucleus of the germ cells. It has also been brought out that spermatocytes are also target cells for androgen in showing 'target tissue metabolism' (Dorrington & Fritz 1973). The ABP thus released may repeat the process or the androgen-ABP complex may be secreted into the lumen of the tubule. There is also a likelihood of the ABP being broken down by proteolytic enzymes within the germinal epithelium. Sanborn *et al.* (1976) do not go beyond the entry of the steroid-receptor complex into the germ cell as to its further fate.

In the diagrammatic representation (figure 24), Steinberger *et al.* (1978) (see also Dorrington & Fritz 1975) have indicated the diffusion of the androgen into the tubule from the Leydig cells; in the Sertoli cell, the androgen is bonded with a cytoplasmic receptor and then the complex translates into the nucleus. A segment of DNA transcribes mRNA, and protein synthesis takes place. There is also a possibility of the cAMP activated protein kinase making an entry into the Sertoli cell nucleus, but the result is unknown. It is known that nuclear androgen receptors are present in Sertoli cells but not in spermatocytes and round spermtids (see Ritzén *et al.*

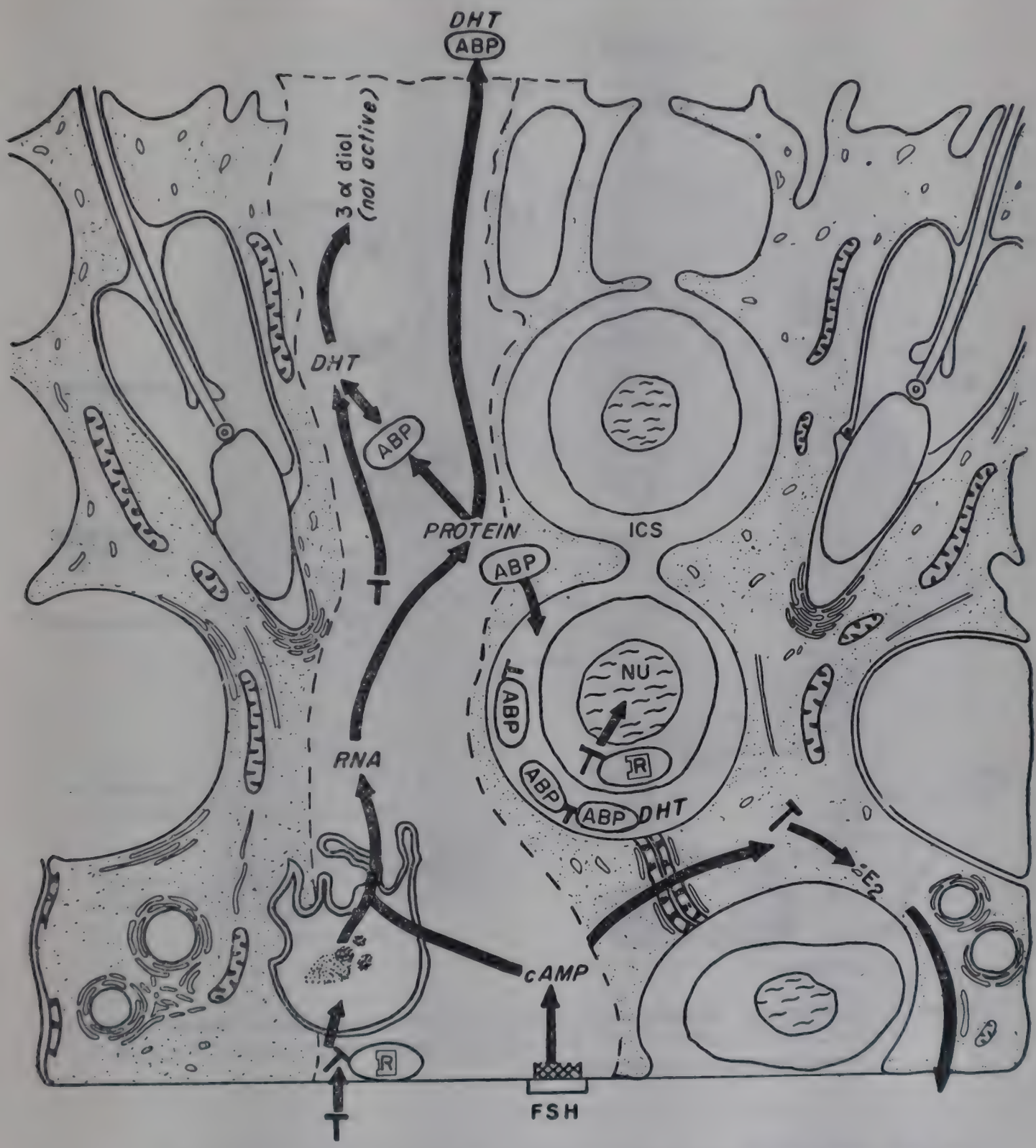


Figure 23. Diagrammatic representation of the action of FSH and androgen on the Sertoli cell, both acting directly on the Sertoli cell, leading to the synthesis and release of ABP and the aromatization of T into OE_2. After activation, the homone-receptor complexes are translocated into nuclei where transcription takes place (after Purvis *et al.* 1977, modified)

1981). Tsai *et al.* (1977) in describing the nuclear acceptor sites do not discuss how the cytoplasmic androgen receptors move into the germ cells.

It has been brought out (Ritzén *et al.* 1981; see their figure 3) that FSH activates

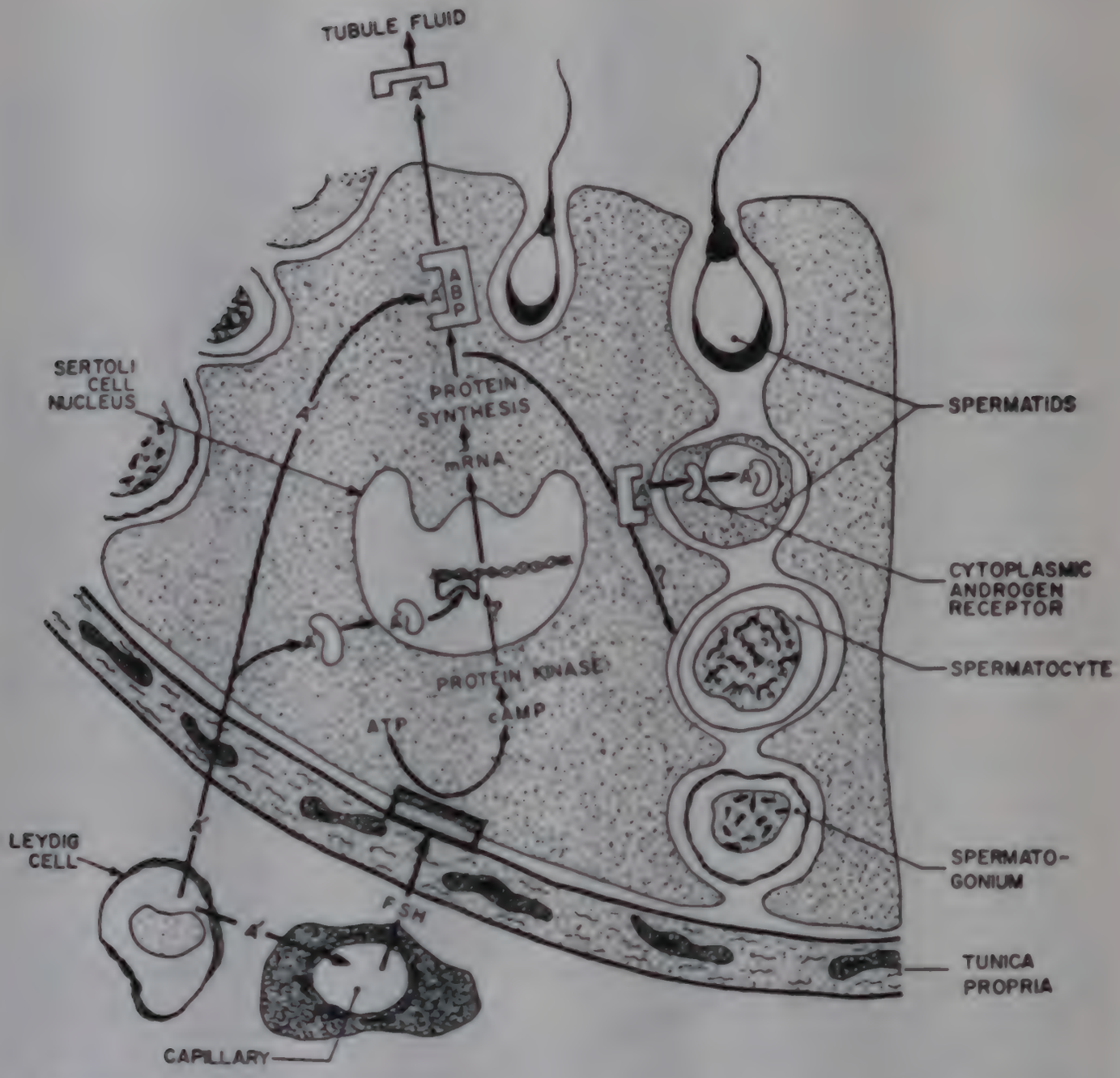


Figure 24. Diagrammatic representation of the action of FSH on the Sertoli cell (after Steinberger *et al.* 1978). Note the entry of T by diffusion into the tubule lumen and the entry of protein kinase into the nucleus (?)

the cAMP in the Sertoli cells and the cAMP also acts as a second messenger causing protein kinase activation and the latter may cause phosphorylation of proteins in the cytoplasm (and the nucleus?). There appears to be production of ABP also. Testosterone which enters the Sertoli cells is bonded to soluble cytoplasmic receptor (either as T or as DHT) which may undergo an allosteric conformational change (their figure 3: $CR^1 \rightarrow CR^2$); this complex enters the nucleus and the transcribed mRNA may give rise to more ABP. This androphilic protein may enter the tubular lumen and/or the intercellular space helping germ cell maturation.

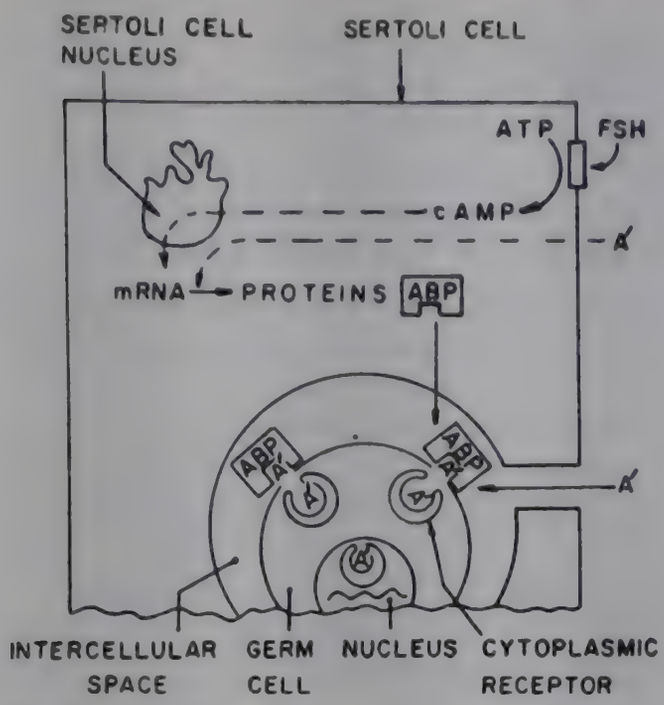


Figure 25. Diagrammatic representation of the action of FSH on Sertoli cell receptor and how the cytoplasmic receptor with androgen enters the nucleus of the Sertoli cell. It is not clear what happens further (after Sanborn *et al.* 1976, modified)

ABP disappears on hypophysectomy but reappears on injection of FSH (Hansson *et al.* 1973b). Purvis *et al.* (1977) quoted that immediately after hypophysectomy, T could not maintain ABP production in the absence of FSH; if there is delay, T cannot initiate it but FSH can at any time after pituitary ablation (Weddington *et al.* 1975c). FSH is necessary for its maintenance of secretion. It is also known that in adult rats, LH can bring about increase in ABP levels (Vernon *et al.* 1974). Weddington *et al.* (1975a) also brought out that ABP was identical histochemically to sex-steroid binding globulin (SBG).

Androgen stimulation is not necessary for ABP production (Ritzén *et al.* 1981); the androgen insensitive testicular feminised male (tfm) rats show high concentration of ABP in the testes (Hansson *et al.* 1978) and these lack efferent ducts and epididymides. Totally androgen resistant 'tfm' mouse also shows testicular ABP.

Castration caused disappearance of ABP and it could not be re-induced by hormonal treatment in rats (Ritzén *et al.* 1981).

Studies so far made with regard to ABP and SBG disclose:

(i) rabbit ABP and its serum steroid (testosterone) binding globulin are indistinguishable, as probably the same gene is responsible for both. However, as rabbit ages, they are regulated differently (Weddington *et al.* 1975b). Tindall *et al.* (1978a), however, argue that it may be that the binding proteins from serum are transferred to the testis with ageing,— a point worth exploring;

(ii) rat ABP and rabbit SBG, and rat ABP and rabbit ABP differ in their steroid specificities;

(iii) preliminary studies of Tindall *et al.* (1978a) have shown that human SBG and rabbit SBG have similar steroid-specificities;

(iv) in man, the plasma shows distinct testosterone- and cortisone-binding globulins (Vermeulen 1973); further, 60% of testosterone is bound to its binding globulin, 40% to albumin, 1% to cortisol binding globulin, and 2% is free (see Setchell 1978).

In the rat, testosterone binding globulin is absent (Ritzén *et al.* 1971) as also in boar (Corval & Bardin 1973) and, therefore, free testosterone is more. In these, the purity of ABP is also not vitiated. But in those species where both occur, it becomes difficult to differentiate as e.g., the ABP in the testis and epididymis of man and monkeys and the serum SBG (Vigersky *et al.* 1976), so much so the presence of ABP in man is even doubted.

Tindall *et al.* (1978a) reported that ABP and SBG were very different from the androgen cytoplasmic receptor (CR). For CR binding, 3-keto and 17 α-hydroxy moieties appear to be necessary and with any modifications in these two, ABP can still show binding. If, however, the 19-methyl group is removed, there is reduced binding for ABP but binding for CR remains unaffected.

Wilson *et al.* (1978) have attempted to give the main characteristics of ABP. It is a medium-sized acidic protein (molecular weight about 90,000) moderately sensitive to environmental changes inclusive of *p*H and heat; like the androgen-receptor and other ABPs, it has a preference to DHT and T over other steroids. It is synthesized in the Sertoli cell and it may accumulate T or DHT there; it becomes an intracellular carrier. Its synthesis is dependent on FSH and androgen (see Ritzén *et al.* 1971; p. 47) and these hormones may act independently but synergically. From the Sertoli cell it is thrown into the tubular fluid wherefrom it goes to the epididymis carrying the androgens and the high concentration of T probably helps in the maturation of sperm. ABP can also bind in the epididymal chamber (caput), the T coming from the surrounding blood. Finally, it may be enzymatically destroyed. Since in man, monkey, bull and swine, there is little ABP in rete testis, transportation function ascribed to it is probably not essential (Ritzén *et al.* 1971).

The antiandrogen cyproterone acetate which is known to inhibit androgen-binding to receptors, appears to have no effect on ABP (Tindall *et al.* 1978a); Purvis *et al.* (1978c) note that cyproterone affects directly the steroidogenic enzymes (3β-HSD isomerases) and not the receptor-adenylate cyclase system.

8e *Cyclic Activity of Sertoli Cell and Spermatogenetic 'Wave'*

In vitro studies of rat spermatogenesis (Parvinen & Vanha Perttula 1972, Parvinen *et al.* 1980) were quite revealing. Stages II–VI, VII–VIII, IX–XII and XIII–XVI–I were incubated for 20 hrs at 32°C; stage VII–VIII was found to secrete 70% more ABP than II–VI and they also showed highest RNA synthesis. When different segments were incubated with FSH and PDE inhibitor, two reactions were noticed:

(i) cAMP levels in stage II–VI showed ten times higher cAMP production than in stage VII–VIII;

(ii) concentration of FSH receptors in II–VI stage was twice that in VII–VIII stage.

In these two features, stage VII–VIII is inversely correlated with stage II–VI. This shows that Sertoli cells very active in protein synthesis and secretion are the least sensitive to FSH-stimulation.

8f *The Cryptorchid Testis*

As early as 1969, Clegg brought out that in cryptorchid rats the high temperature brought about the failure of spermatogenesis but the Sertoli cells were unaffected. Using ABP as a marker of the Sertoli cell, it was found that the cells were affected in cryptorchidism. Adult rats were made cryptorchid or testes were prevented from descending into the scrotum. Two important facts emerged from these studies:

(i) the level of androgens and FSH in the interstitial fluid surrounding the tubules was higher than normal (Hagenäs *et al.* 1978a);
(ii) androgen-receptor content of cryptorchid testes of hypophysectomised rats was the same as that of noncryptorchid hypophysectomised rats.

There was, however, a decrease of testicular receptors for FSH in cryptorchid rats. Since ABP is impaired in Sertoli cells when temperature is raised from 32 °C to 37°C in the absence of hormones, probably nonhormonal factors are involved in this (Hagenäs *et al.* 1978b). The blood-testis barrier is normal in cryptorchid rats. It is not clear if the impairment of Sertoli cell activity is the cause of failure of spermatogenesis.

8g *Androgen-synthesis and the Sertoli Cells*

The testis produces a number of androgens which are biomessengers throughout life (Rosness & Eik-Nes 1977). It is the intertubular interstitial cell or Leydig cell which synthesizes testosterone (see figure 24) and this may pass into the tubules by blood or lymph or may rapidly diffuse into the tubule through its walls. The membrane-bound enzyme 5 α-reductase catalyses the conversion of testosterone into 5 α-dehydro-testosterone (DHT) in the testis of rat (Rosness & Eik-Nes 1977); according to the same authors, the enzyme is also present in the interstitium. In the rat, the active conversion seems to take place in the immature testis (30–40 days) and it is also during this time that some of the spermatogenic stages are being established (Rivarola *et al.* 1972). DHT does not seem to play a major role in the maturation of the testis as it is only produced in minute quantities at maturity (Folman *et al.* 1972). Both testosterone and DHT can be synthesized by the testis starting from acetate.

Though seminiferous tubule showed small concentration of testosterone, a *in vitro* culture of the seminal tissue did not synthesize the androgen (Ewing & Brown 1977). All the same it appears to have the capacity to concentrate the androgen for local action; as already said, the disposition of SER close to the acrosome of the developing spermatid in the Sertoli cell may be for the elaboration of some lipid or steroid factors acting on the differentiation of the acrosome cap (Fawcett 1972). The steroidogenic enzymes are sequestered in the mitochondria or endoplasmic reticulum i.e., within the membrane of this reticulum (Ewing & Brown 1977, Ewing *et. al.* 1980).

The androphilic protein may bind both testosterone and DHT. The ABP can bind itself to DHT with 2–3 times greater affinity than it does to testosterone (Hansson *et al.* 1973a). According to Hansson *et al.* (1976), the DHT dissociates itself from ABP very quickly and the latter functions more like a carrier or a transport protein. SBG can also bind DHT with great affinity (Vigersky *et al.* 1976). While 5 α-dehydrotestosterone exhibited highest affinity for ABP, 5 β-dihydrotesto-

sterone had little or no affinity for the binding site (Tindall *et al.* 1978b). Proteolytic enzymes appear to digest DHT. French and Ritzén (1973) opined that ABP at the site of production viz., the Leydig cells, may help in transporting testosterone from the Leydig cells to the tubule. Further, Hansson *et al.* (1973a) stated that ABP within the tubules generates a diffusion potential causing a net inflow of testosterone. It is rather intriguing to note that no sooner ABP is synthesized in the Sertoli cell, it is secreted into the lumen (Tindall *et al.* 1974); and if so, how can it accomplish the binding functions attributed to it?

In addition to these two important androgens, there are also others in the testis. Figure 23 shows an inactive androgen 3α-diol (5α-androstane, 3α, 17β-diol) (= DIOL) towards the luminal side. According to Dorrington and Fritz (1975), DHT can be metabolised into 3α-diol in the Sertoli cell; both spermatocytes and Sertoli cells can convert testosterone into DHT; in the spermatocytes the 3α-hydroxysteroid dehydrogenase activity is low. Such biotransformation of testosterone into DHT is not possible in the spermatid (Dorrington & Fritz 1975). Mature testits can convert inactive 3β-diol to DHT and thus into 3α-diol according to unpublished report of Sunde, Rosness and Eik-Nes (see Rosness & Eik-nes 1977). This only means that testosterone metabolic-enzymes can transform testosterone into suitable hormones according to the needs of the seminal cells.

Testosterone on becoming intracellular in Sertoli cell is reduced to DHT (a bioactive form of the androgen) by 5 α-reductase in immature testis; in the maturing one, the enzyme is cosiderably reduced both in the tubule and interstitial tissue (Hansson *et al.* 1975) and there is accumulation of T. The DHT formed appears to be bound by cytoplasmic receptors (CR) which completely encompass the steroid moiety and therefore the dissociation is poor (see figure 24; also Engel L L (1967), quoted by Purvis *et al.* 1977). These cytoplasmic receptors are very different from ABP as for example the CR's mol. weight is 200,000 while that of ABP is 90,000. The, thus, receptor complex-bound DHT enters the nucleus of the germ cell (Dorrington & Fritz 1975); this mode of translocation of the cytoplasmic androgen-receptor complexes into nuclei appears to be common in all target cell responses. It was experimentally proven that chromatin of Sertoli and germ cells exhibits capacity to interact with androgen-receptor complexes. This activation probably brings about events in the cell and it is yet to be proven that they are linked to transcriptional and translational sequences (Steinberger *et al.* 1978).

Referring to antiandrogens it is found that conversion of T into DHT is not reduced by an androgen antagonist like CA (Belham & Neal 1971); the drug can completely inhibit bonding of DHT by the epididymal cytosol (Ritzén *et al.* 1971).

It may be that both T and DHT are bound by the same CR in which case, DHT occupies a deeper position and T a superficial one when the latter can be stripped by specific antibodies. It is noticed that T not only acts on the nucleus but also activates the turn over of endoplasmic reticulum, the physico-chemical structure of protein biosynthesis and the activity of the Golgi complex (Dym & Fawcett 1970).

The T in addition to entering directly into Sertoli cell may also enter the tubule lumen by passing in between two Sertoli cells through the tight junctions which selectively allow it. In the lumen, the androgens are bound to ABP; this metabolism

helps the testis in spermatogenesis [and in the epididymis in sperm maturation, Hansson *et al.* (1975)] and it may also act as a protection against enzyme action (Dym & Fawcett 1970).

That the Sertoli cell is the target for both FSH and testosterone in the tubule is well established (Steinberger *et al.* 1979). The Sertoli cell probably mediates the effect of these two hormones on the germ cells. This has been brought out by Lyon *et al.* (1975)] in chimeric mice where they showed the germ cells lacking androgen receptors do develop into fertile spermatozoa in the presence of normal Sertoli cells.

8h *First Appearance of ABP in the Testis*

This protein appears in the testis even before the lumen appears in it (Vitale *et al.* 1973); both the lumen and the Sertoli-Sertoli cell tight junctions appear almost together (16–18 days postnatally in rat). In 15-day-old rats, where there is no lumen in the testis, ABP is met with in the Sertoli cell. The epididymis is free of it. In Sertoli cell-only rats ABP appears on the 15th day; it goes on increasing till 29–30 days when there is a sudden drop of it in the testis; the epididymis showed the presence of it now. It must have moved into the epididymis along with the testis fluid. Judging from the ABP levels in epididymis, Tindall *et al.* (1975) described that the Sertoli cells appear to be morphologically fully differentiated by 50–60 days. Along with the androgens which ABP binds, it moves into the epididymis from the seat of formation, viz., the Sertoli cell. Hansson *et al.* (1973c) reported that in the rat the epididymis was divided into five segments; segment 1 was the caput where the greatest concentration of ABP (and also DHT) was noticed. Its binding capacity decreased from segment 1–5. The bound androgen in a concentrated form plays upon the sperm bringing about maturation; it also appears to play upon the epithelial cells.

8i *Immunization Studies on Gonadotropin Antisera*

Spermatogenesis and steroidogenesis, the two important facets of testis physiology, have been studied vis-a-vis hormonal control. As early as 1936, Greep and his coworkers put forward the dual hormonal control of testicular function,viz., FSH controlling spermatogenesis, and LH, androgen synthesis. It was, however, brought out by Steinbergers (1974) that in hypophysectomised rats,T alone could maintain spermatogenesis and therefore, the role of FSH became dubious. It is in this connection that immunological studies have been very helpful when purified gonadotropins became available and specific antiserum for either FSH or LH could be prepared and it selectively knocked out either FSH or LH and enabled to study the effect of the remaining gonadotropin. This obviated hypophysectomy.

In the rat using passive or active immunization against LH leads to reduction of testicular size, derangement of spermatogenesis, and atrophy of accessory glands (Moudgal & Li 1961; Talaat & Laurence 1971). Passive immunization of adult rats against FSH made them less fertile but there was no reduction in testis weight. Madhwa Raj *et al.* (1980) have used both rat and crab-eating monkey (*M. fascicularis*) for studying FSH antisera action. They have found that in the rat, FSH was not

necessary for the maintenance of spermatogenesis once it started. In the monkey, on the other hand, FSH appears to be necessary in the adult for the maintenance of spermatogenesis. In the bonnet monkey, passive immunization with FSH antiserum (produced in monkeys) brought about inhibition of spermatogenesis, low levels of sperm count and decreased hyaluronidase level in semen. Active immunization in the crab-eating monkey also caused similar results. While these changes were noticed in immunization against gonadotropins, the authors state that T level was the same in the two monkeys studied.

A number of experiments has been done for studying the effects of antisera of gonadotropins on the morphology and biochemistry of Sertoli cells. Using 33-day-old rats in which the Sertoli cells resemble adult ones, and adminisiering antisera for FSH (AS-FSH) or LH (AS-LH) or AS-LH plus testosterone propionate (TP), the Sertoli cells reacted differently (Chemes *et al.* 1979). With AS-FSH, there was a dilatation of the endoplasmic reticulum and the ABP levels in the testis and epididymis decreased. With AS-LH, the nuclei were reduced, the nuclear membrane was smoothened out and the number of nucleoli was also reduced. Cytoplasm showed fewer mitochondria, Golgi and endoplasmic reticulum. ABP was reduced even more than in AS-FSH treated ones. Addition of TP to AS-LH group brought back the infolding of nuclear membrane and the number of nucleoli. No change was noticed in the ABP level. It is also stated by the authors that the blood-testis barrier formation may be reduced or even absent in the AS-LH treated group.

When immature male rats (20 days old) were treated with AS-LH over 14 days (Madhwa Raj & Dym 1976), the testes lost 80% of their weight; the accessory glands also lost weight and serum T level was very much lower than in the controls. AS-FSH while causing loss in weight of the testis (50%), it did not affect the accessories or the testosterone level. This clearly indicates that selective FSH depletion interferes with spermatogenesis in immature rat.

9 INHIBNI AND ITS ROLE

It is common knowledge among reproductive biologists that the mammalian testis secretes a protein hormone in addition to steroid ones. A short historical account may not be out of place. More than 50 years ago, x-irradiation experiments showed in rats, the seminiferous tubules were shrunken and disorganized; the Leydig cells were normal. The pars anterior of the pituitary gland showed castration changes. It was inferred that a seminiferous tubule hormone brought about a feedback inhibition of the pituitary gland (Mottram & Cramer 1923). This negative feedback hormone was called Andrhormon or Andrins by Martins and Rocha (1931); McCullagh (1932) called it inhibin as it had an inhibitory property and the same name is retained today. Later Klinefelter *et al.* (1942) called it X-hormone.

Further research was bedevilled for want of a good bioassay for this second hormone. Two hypotheses sprang up in connection with the feedback action:

(i) *The inhibin hypothesis*: Both FSH and LH act on the testis. LH causes androgen (testosterone) secretion from the Leydig cells; this androgen has a negative feedback effect on the interstitial cells. Similarly FSH acts on

the seminiferous tubules and causes (a nonsteroidal) hormone inhibin to be secreted, and it has a negative feedback effect on the FSH secretion.

(ii) *The androgen hypothesis*: The steroid secreted by the testis has a negative feedback effect on both FSH and LH. The snag about the second hypothesis is that no steroid is known to suppress both FSH and LH in castrated animals. Many androgens do suppress FSH but not LH. A single steroid suppressing both FSH and LH has not been found. Under these circumstances, inhibin hypothesis becomes more attractive. With all this work having gone into inhibin studies, Main *et al.* (1979) are constrained to note that a reciprocal relationship between FSH-secretion and inhibin-production has not been convincingly demonstrated. Inhibin probably plays a minor role in the male, and it may be a local one for causing maturation of spermatogenic cells. The negative feedback in that case is taken over by the androgen.

Intensive research to characterise this hormone, which has a controlling effect on the pituitary FSH-secretion, was started and different parts of the testis were examined. The presence of inhibin in the rete testis fluid was first reported by Setchell and Sirinathsinghji (1972) and Setchell and Jacks (1974). It was also discovered in the spermatozoa (Lugaro *et al.* 1973, 1974), seminal plasma (Franchimont *et al.* 1975a, 1975b, 1975c, Chari *et al.* 1978, Vaze *et al.* 1979), testis (Lee *et al.* 1974, Keogh *et al.* 1976, Moodbidri *et al.* 1976), ovary (Hirschhauser *et al.* 1976) and follicular fluid (Hopkinson *et al.* 1975, de Jong & Sharpe 1976, Welschen *et al.* 1977, Marder *et al.* 1977, Schwartz & Channing 1977, Daume *et al.* 1977). Franchimont *et al.* (1975c) reported that inhibin may not only control spermatogenesis but it may also help in the oestrous cycle. Franchimont *et al.* (1978) have brought out that in their *in vitro* experiments, and those of Baker *et al.* (1976), inhibin reduced secretion of both FSH and LH consequent on receiving LRH. Inhibin does not affect the release of TSH, GH or prolactin both *in vivo* and *in vitro* (Franchimont *et al.* 1978). Steinberger and Steinberger (1977b) even postulate that the synthesis and release of gonadotropin may be affected by inhibin. Setchell *et al.* (1977) reported that inhibin could not be a steroid, as steroids tested either reduced serum FSH and LH together or reduced LH but did not affect FSH. It is definitely a protein; its activity is destroyed by heat and proteolytic enzymes and not by steroid solvents. Its molecular weight is between 15,000 and 70,000 daltons. Inhibin protein is also secreted by the Sertoli cell but is very different from the androgen-binding protein. If Sertoli cells are cultured with pituitary cells *in vitro*, the output of FSH is reduced. According to Nandini *et al.* (1976), it is a heat-stable, lyophilizable and non-dialyzable material. Chari *et al.* (1976) have put forward a bioassay for inhibin. It is based on a dose-dependent suppression of hCG-induced ovarian weight increase in intact, immature female rats.

Inhibin appears to act both on the hypothalamus and pituitary gland. In young rats and hypophysectomised adults (Setchell *et al.* 1977), FSH appears to have a number of actions which could be put under two heads: (i) causes production of ABP from Sertoli cells, and (ii) increases testis weight, tubule diameter, and the number of pachytene spermatocytes per tubule cross section. There is no reference in literature so far that in the adult animals FSH controls quantitatively

spermatogenesis. If there is, then inhibin would be the agent for feedback quantitative information on the rate of sperm production.

Lee *et al.* (1979) have put forth studies towards providing a suitable bioassay for inhibin, the object being to use this hormone as a suitable male contraceptive; it is known to have a negative feedback effect on FSH. In their studies of *in vivo* assays, the acutely castrate mouse assay system was very sensitive; one unit of inhibin activity suppressed 50% of the post-castration rise in FSH. They, however, feel that *in vivo* bioassays are not sufficiently sensitive. They have developed a *in vitro* dispersed pituitary cell culture system which is precise and sensitive. The gonadal steroids oestradiol and testosterone have no inhibitory effect on pituitary FSH responsiveness to LHRH. This may lead to the purification of inhibin.

Shashidhara Murthy *et al.* (1979) described that purified sheep testicular inhibin significantly suppressed plasma FSH level in long term castrated bonnet monkeys. It also works in mouse and rat and therefore, it is not species specific.

The French workers Claire Cahoreau *et al.* (1979) compromise the *in vivo* measurements (Setchell *et al.* 1977) which consider inhibin as suppressing FSH and *in vitro* (Baker *et al.* 1976) where suppression of LH response to GnRH is used to measure inhibin and state that the protein inhibin suppresses both plasma FSH and LH levels.

To study the inhibitory effect of FSH on spermatogenesis in adult rats, male rats were actively immunized for 53 or 116 days. At the end of the periods when LH and testosterone were assayed by RIA, there was no difference with control rats. It is likely that libido and potency are not impaired in these experimental animals. There was, however, diminished spermatogenesis; the number of primary spermatocytes and early spermatids was lower in 53 days experimental rats while late spermatids were lower in 116 day experimental ones. A complete inhibition of FSH by inhibin was never realised *in vivo*. Since inhibin impairs spermatogenesis, it can be used as a potential male contraceptive.

Two diagrammatic representations (figures 26, 27) are reproduced; one is from Setchell *et al.* (1977), based on a diagram of Fawcett (1975a). Blood borne hormones have a negative feedback on the hypothalamus and hypophysis controlling LH and high levels of FSH synthesis. The inhibin from Sertoli cells may feedback on hypothalamus and hypophysis to control FSH production and at high levels also LH synthesis. In another diagram (figure 27), Gupta (1977) has brought out that in addition to LH and FSH, prolactin and growth hormone also appear to affect the testis and as before the androgen in blood may feedback on the pituitary and hypothalamus.

As recently as 1977, the Steinbergers (1977b) raised two important questions regarding inhibin (Sertoli cell factor). It is known that it has a negative feedback effect on the pituitary, suppressing FSH but not LH secretion. The first question refers to how exactly does inhibin reach the pituitary to bring about a selective action? In answer to this question, Le Lannou *et al.* (1979) have brought out that the inhibin along with the testis fluid passes into the initial lobe (caput) of the epididymis. Here it is absorbed and passes into the blood stream from where it may go directly or through the hypothalamus to the pituitary (Le Lannou & Chambon 1977). The second question is the role of gamma-glutamyl transpeptidase

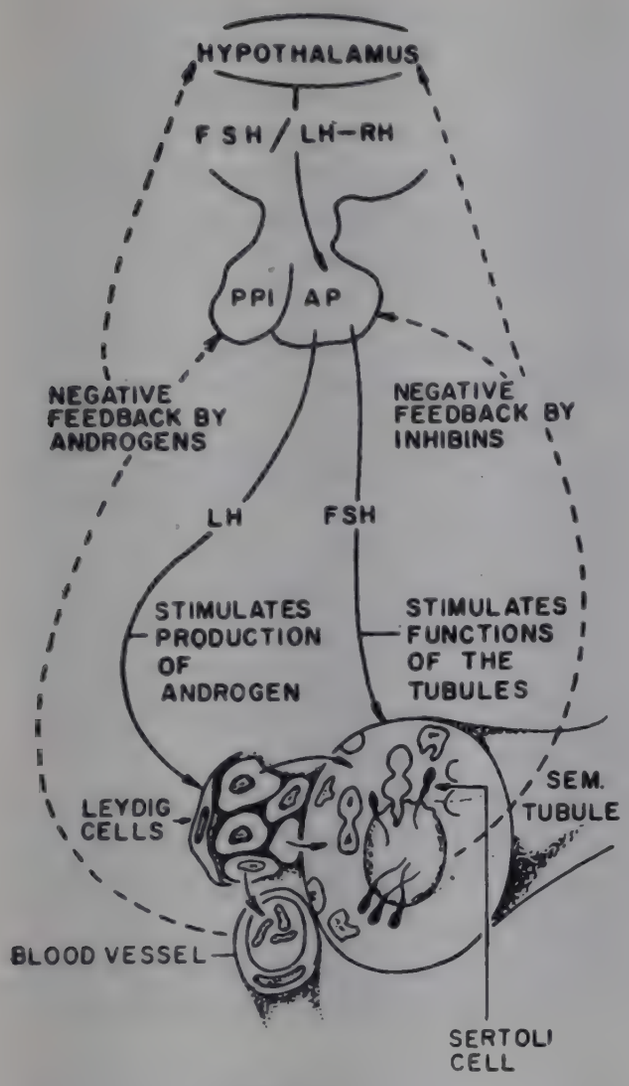


Fig. 26

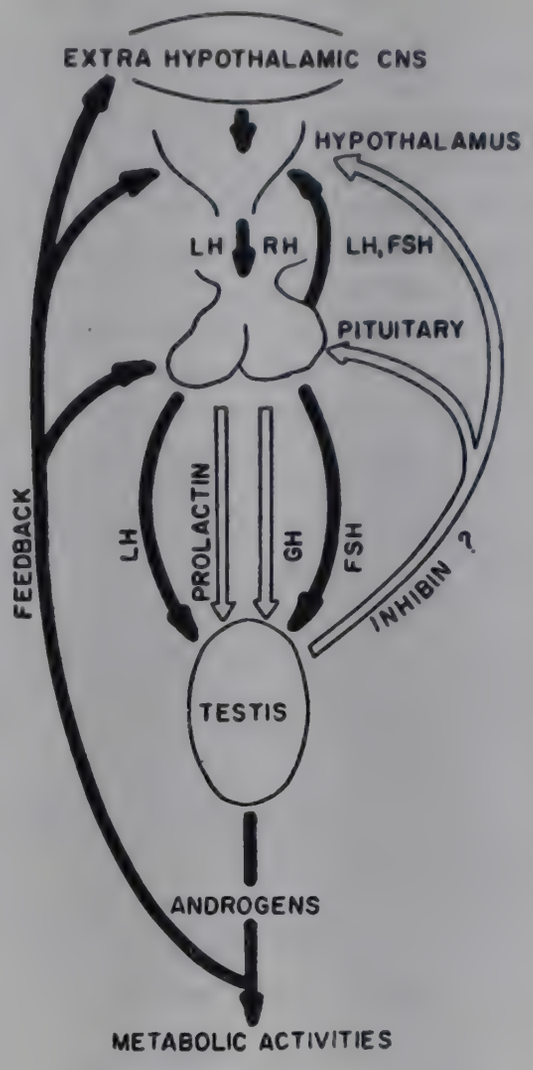


Fig. 27

Figure 26. Diagrammatic representation of the hypothalamo-hypophysial-testis axis and the negative feedback mechanism of inhibin; the feedback arrow is shown towards the pars nervosa in the original figure (after Fawcett 1975a)

Figure 27. Diagrammatic representation of the hypothalamo-hypophysial-testis axis and the negative feedback mechanism of inhibin. Growth hormone and prolactin are also involved; the feedback arrow is shown towards the pars nervosa in the original diagram (after Gupta 1977 modified)

enzyme which helps in protein synthesis. Does it also help in the synthesis of ABP and inhibin in the Sertoli cell? It may be mentioned that GTP is present only in the Sertoli cells in the seminiferous epithelium, it can function as a potential marker of it (Hodgen & Sherins 1973); whether GTP helps in the synthesis of ABP and inhibin is still unclear.

10 THE MORPHOLOGY AND PHYSIOLOGY OF THE LEYDIG CELL

10a *A Historical Account*

It would be no exaggeration if one says that the mammalian testis is composed of two anatomically and functionally distinct compartments. They are the seminiferous

tubules and the Leydig or the interstitial cells. The first is responsible for spermatogenesis while the latter is the factory where androgens (testosterone, DHT) are synthesized. Biochemically the two are linked,—without androgen, spermatogenesis is not possible. It may also be pointed out that the biosynthetic enzymes for prostaglandins (PGs) hormone synthesis are related to steroid biotransforming enzymes and the PG synthetase activity is seen in the interstitial cells and the mature germs cells of the seminal tubules. The testis can therefore be described as a dual endocrine gland (Ellis 1972a, 1972b).

Historically, in a very suggestive experiment conducted by Berthold (1849) by transplanting testis ectopically into castrated roosters, he found that the combs did not atrophy. This allayed all previous views and it was inferred that the blood regulated the relationship between the testes and the accessory glands. A year later, Franz Leydig (1850) described the interstitial cells in the testis and various functions were attributed to them. By 1930, it came to be known that the male hormone was testosterone and that the pituitary hormones controlled the testes function. In hypophysectomised animals, the luteinising hormone of the pituitary gland not only reactivated the Leydig cells but also the male characteristics. In mice it was noticed that the Leydig cell tumours produced high level of androgen. The inference was that Leydig cells were the source of testosterone.

It was the discovery of a histochemical technique for identifying the steroidogenic enzyme 3 β-hydroxysteroid dehydrogenase (3 β-HSD) in mammals which proved that Leydig cells had an abundance of it. Further, when radioactive progesterone was incubated with Leydig cells it was found that this substrate was converted into androgen. It is now known that most of the oestrogen found in the testis arises from the aromatization of testosterone into oestradiol 17β by the Sertoli cell (Dorrington *et al.* 1978b).

For the development and maintenance of Leydig cells, fetal pituitary gland is necessary; anencephalic embryos lack Leydig cells (Steinberger & Steinberger 1972).

10b *Morphology of the Leydig Cells*

The Leydig cell in addition to synthesizing androgens which enter into general body circulation or directly into testis tubule, also carries on the routine functions of the cell. The cytoplasm shows lysosomes. The cell may contain different hydrolases (some 3 dozens) (primary lysosomes); these may fuse with the material to be broken down or with phagocytic vacuoles (secondary lysosomes). When the material has been digested, the resulting body is called a 'residual body' or 'dense body'. The term 'residual body' is not a happy one since the same term has been used to designate the cytoplasmic body left behind when the sperm escape into the tubular cavity from the Sertoli cell. A single membrane bound peroxisome is another microbody occurring in the Leydig cell. What exactly is the function of this body in the steroid secreting cell is unclear.

Between the Leydig cells, their plasma membranes may come in contact exhibiting gap junctions and even a few punctate tight junctions are reported in guinea pigs of 25–26 days of age; they appear to increase with age (Black & Christensen 1969, see their figure 10).

10c *Origin of Interstitial Tissue*

The interstitial tissue arises from the gonadal mesenchyme. Morphogenesis sets in the testis after some time and the interstitial cells change; the cell size increases and the shape becomes oval from elongate. These now get to be called immature Leydig cells. In them, smooth endoplasmic reticulum appears, mitochondria become numerous while ribosomes and rough endoplasmic reticulum decrease. The fetal Leydig cells also undergo regression noticed in laboratory rodents like mouse, rat, hamster and rabbit but not in the guinea pig. Gondos (1977) considered it to be no regression but a reversion to a less well differentiated condition or a quiescent state noticed in the postnatal prepubertal period.

10d *Appearance of Testosterone in Leydig Cells*

Moon and Raeside (1972) brought out that as soon as the fetal Leydig cells mature, the appearance of 3β-hydroxysteroid dehydrogenase activity is noticed and androgen production ensues. It is noticed that as soon as the Leydig cells are differentiated in the fetal testis, initial androgen synthesis also takes place (table 1). In the rabbit, postnatally fully differentiated Leydig cells appear at 35 days.

Baillie *et al.* (1966) provide two graphs (figures 28, 29) in one of which they show the age of appearance (figure 28) of the different hydroxysteroid dehydrogenases in the developing mouse testis; in the other (figure 29), they indicate the 12 reactions to reach the testosterone stage starting from cholesterol and both these graphs are reproduced by Blackshaw (1970). Baillie *et al.* (1966) described that reactions 1–4 are caused by 3 β-hydroxysteroid dehydrogenase; reactions 5–8 by 17β-HSD; reactions 9–10 by 3 α-HSD and reactions 11–12 by 11β-HSD. All the 12 reactions take place within the Leydig cells.

10e *The Intertubular Tissue*

The intertubular tissue in which the Leydig cells are laid, has fibroblasts, macrophages and also mast cells (man); blood vessels and lymphatics are invariably associated with Leydig cells. These vessels play an important role in transporting the protein-carrier bound testosterone synthesized in the Leydig cells; the capillary endothelium does not act as a barrier to testosterone so much so, the tubule wall is bathed internally and externally by testosterone (Dym & Fawcett 1970). The

Table 1. *Timing of testicular differentiation, Leydig cell formation and initiation of testosterone synthesis in fetuses of different species*

Species	Testicular differentiation	Leydig cell formation	Initial testosterone synthesis	Authority (not exhaustive)
Rabbit	15-16 days	18 days	18 days	Gondos & Conner (1973).
Rat	13 days	14-15 days	15.5 days	Warren *et al.* (1973).
Mouse	11-12 days	12 days	12.5 days	Russo & de Rosas (1971).
Hamster	11-12 days	12-13 days	—	Gondos *et al.* (1974).
Guinea pig	21-22 days	22-23 days	22-24 days	Price *et al.* (1971).
Human	6 weeks	8 weeks	8 weeks	Siiteri & Wilson (1974).

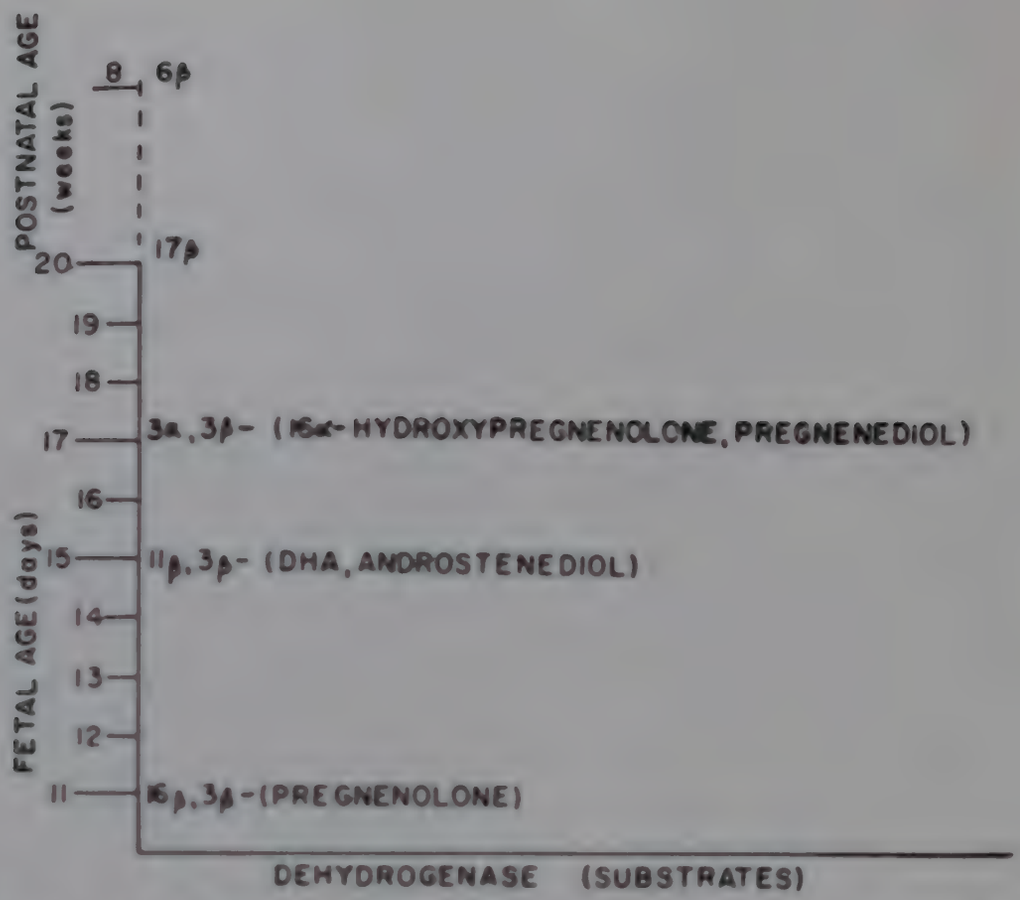


Figure 28. The age of appearance of different hydroxysteroid dehydrogenases in the development of the mouse testis (after Baillie *et al.* 1966, modified)

androgen may enter the tubule through the myoid cell or the peritubular cell layers in its wall.

The Leydig cells have never been seen in division but on LH stimulation, their number increases, probably cells come from some other source. Fibroblastic cells or peritubular myoid cells (human) or macrophages have been implicated as candidates for transformation into Leydig cells. Unfortunately, neither FSH nor LH binds to isolated peritubular cells (Steinberger *et al.* 1975).

10f *Variations in Interstitial Cell Disposition*

The interstitial tissue shows considerable variation in mammals. Fawcett *et al.* (1973) and Fawcett (1973) have given a comparative account of it summarised below:

(i) In the guinea pig, rat, mouse (figure 30a) etc., the Leydig cells are few, the connective tissue stroma is also little but the peritubular lymph channels are many. In the guinea pig, the lymphatic endothelium surrounds the tubules and the perivascular Leydig cells (figure 30a). In the rat and mouse, the lymphatic endothelium is discontinuous and the Leydig cells are bathed by lymph (figure 30b).

(ii) Groups of Leydig cells are found in the intertubular space interspersed with hypertrophied connective tissue cells with a central lymphatic vessel (bull, monkey, man) (figure 30c).

(iii) Numerous closely associated Leydig cells with little connective tissue and lymph vessels (boar, zebra, naked mole rat) (figure 30d).

In the boar and the stallion, the abundance of Leydig cells may in addition be responsible for oestrogen secretion, the exact purpose of which is unclear (Fawcett *et al.* 1973). According to the same authors, occurrence of large number of Leydig cells may also produce steroid olfactory signals [in the boar, $\triangle^{16}$-androstenone is one such compound (Neaves 1975)]. The human testes produce daily about 50 μg of oestrogen of which 10–15 μg are secreted by the Leydig cells while the rest is the effect of peripheral (or extratesticular, Hagenäs 1977) aromatization of testosterone. This large quantity of oestrogen is probably helpful in the feedback regulation of gonadotropins; it is known that oestrogen is more effective in suppressing LH than androgen (Davidson *et al.* 1977).

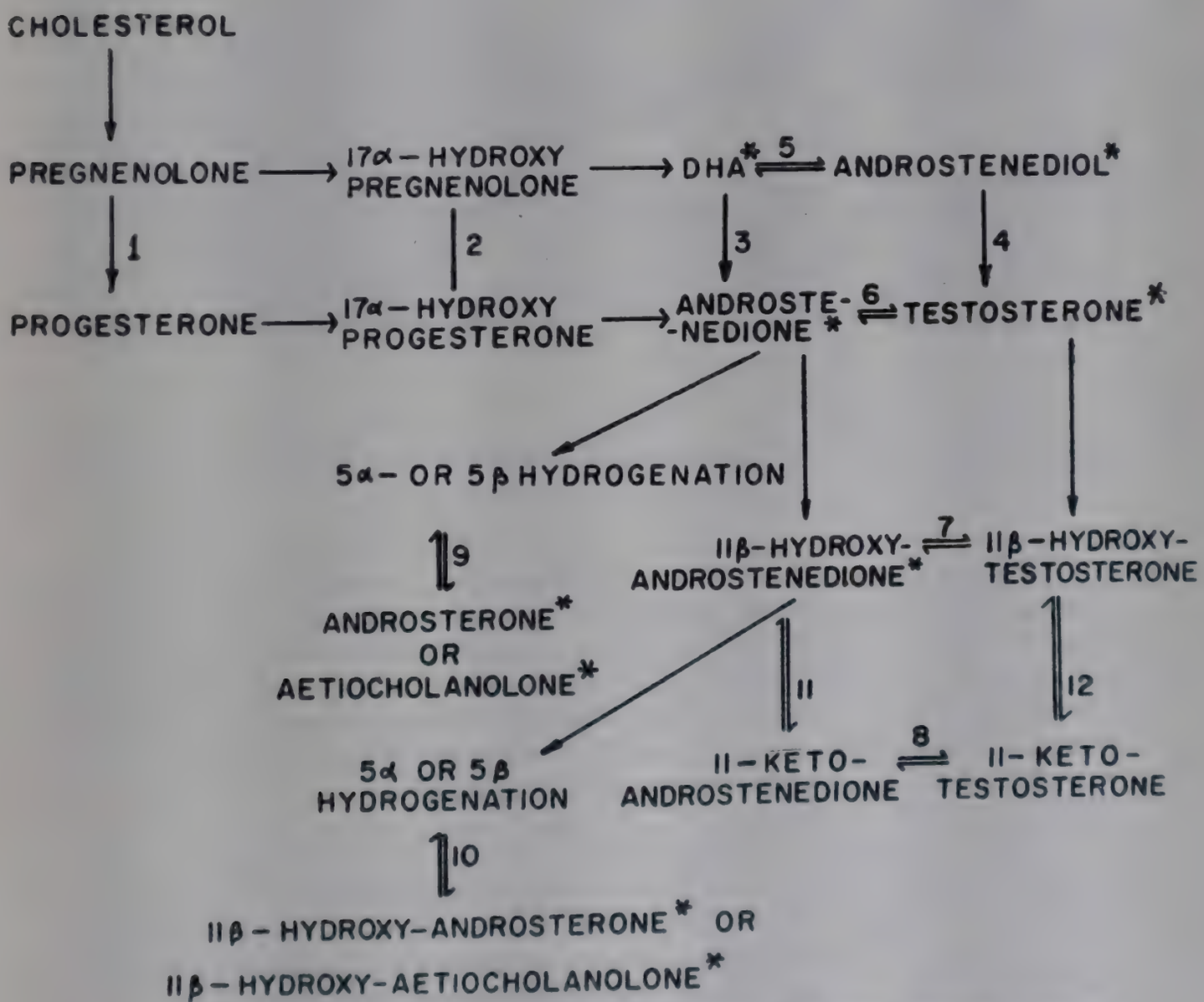


Figure 29. Scheme of steroidogenesis in the testis (after Baillie *et al.* 1966, modified) (Asterisk indicate steroids known to be synthesized in the testis)

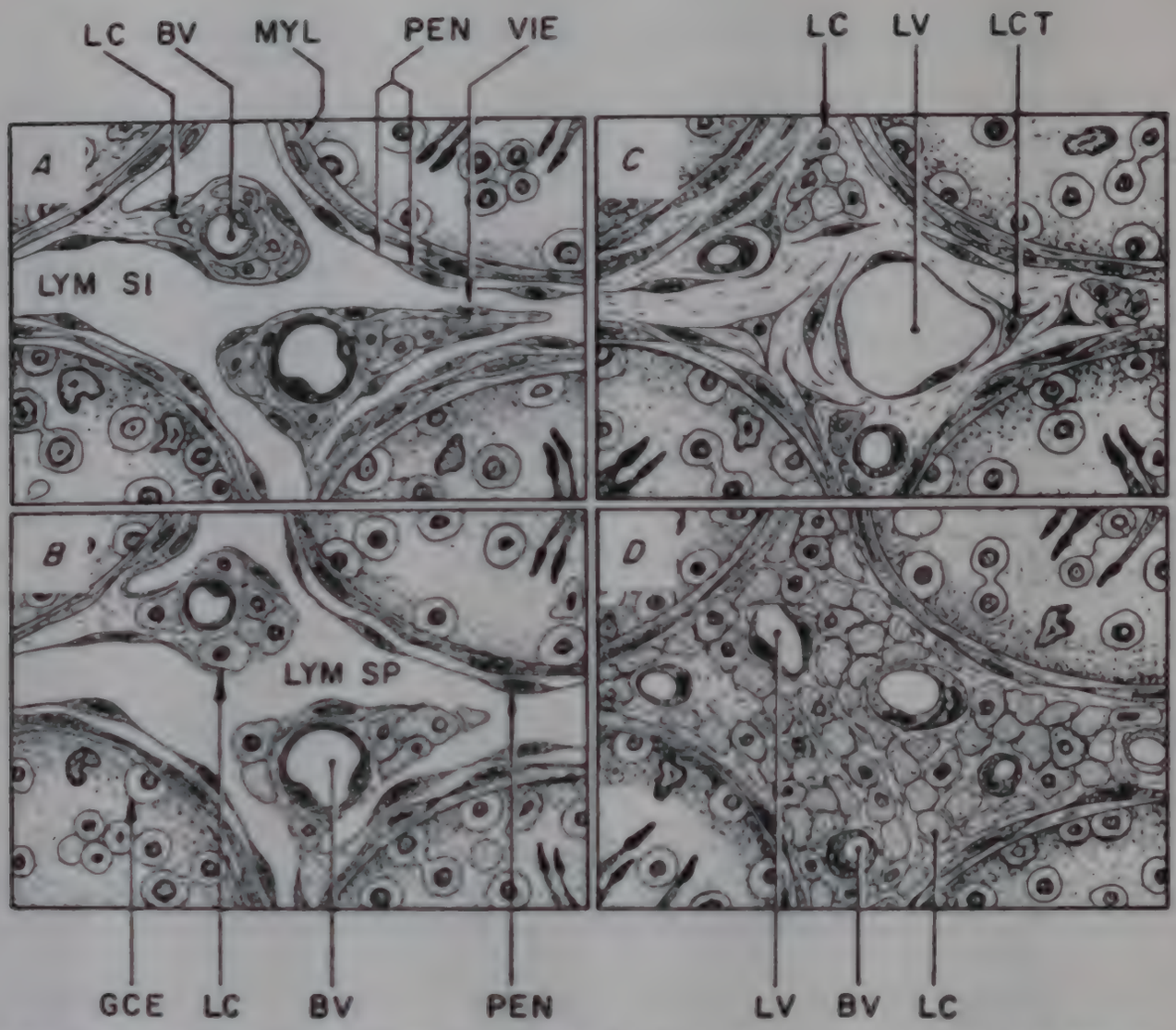


Figure 30. Disposition of interstitial elements in the testis of different mammals (after Fawcett 1973, modified)

It may not be out of place here to point out some situations very different from what has been described above. In the lizard *Cnemidophorus*, the Leydig cells form a highly vascular zone below the tunica. The internal space is reduced to the minimum and a few Leydig cells are also present here. Currie and Taylor (1970) brought out that the enzyme 3β-HSD is present in these two areas and androgen production takes place in them; they also showed that 3β-HSD was absent in the germinal epithelium. Whether a vascular network also is connected with the intertubular space is unclear.

Equally interesting is the fact that in the testis of the squirrel monkey (*Saimiri sciureus*), the interstitial cells are located abundantly on the inner aspect of the tunica albuginea (Belt & Cavazos 1971); the monkey is a seasonal breeder and it is not known what happens to these cells during the non-breeding season. During the period of regression the Leydig cells show abundant agranular endoplasmic reticulum and they anastomose; the mitochondria show tubular and lamellar cristae; there is a Golgi apparatus with associated vesicles and lipid

droplets. These features characterise a secreting cell and it is not clear what happens to the Leydig cell during the regressed period.

The Sertoli cells of the seminal epithelium have been implicated in the synthesis of T. Lofts (see Discussion, p. 30, Hansson *et al.* 1976) described in the soft-shelled turtle, a seasonal variation of spermatogenetic activity out of phase with that of Leydig cells. The seminal tubules show full spermatogenesis but Leydig cells are poorly active or *vice versa.* During spermatogenesis, there is 3β-HSD activity in the Leydig cells; the latter and the accessory glands are both atrophic at this time. With the resumption of activity in the Leydig cells, 3β-HSD activity is seen in them and there is no enzyme activity in the seminal tubules. Isolated tubules were able to convert pregnenolone into T and this was stimulated by FSH and LH.

Courrier (1926, 1927) described a 'eunuchoid' bat (*Pipistrellus*) with full spermatogenesis but regressed Leydig cells and accessory sex glands.

10g *Major Function of the Leydig Cells*

A major function of the Leydig cell is to synthesize T, some 98% of the male hormone (Hagenäs 1977), and this is stimulated by the gonadotropin LH. According to Christensen (1975), the synthesis of T starts from cholesterol. The latter may be drawn from the plasma or it may be synthesized *de novo* in the smooth endoplasmic reticulum by the acetate or from the cholesterol that has been esterified and stored as lipid droplets unbounded by unit membrane in the Leydig cell (Neaves 1977a, b). All the biochemical changes occurring in the Leydig cell have been elegantly brought out by Purvis and Hansson (1981) in their figure 1 which is reproduced by me (figure 31). Numerals used in their figure to represent events are also followed by me. The equilibrium between free (chol) (figure 31, numeral 6) and esterified cholesterol (cholest) s probably influenced by prolactin (PRL) and prostaglandins. The newly-synthesized proteins (figure 31, numeral 5) in the Leydig cell may split the cholesterol esters and a metabolically active cholesterol is released; further, a soluble esterase in the cytoplasm may hydrolyse the cholesterol ester in the lipid droplet and this may yield more free cholesterol. In all these three cholesterol mobilizations, the free cholesterol has to enter the mitochondrion (helped in this probably by a receptor or a carrier protein (figure 31, numeral 7) for its side-chain cleavage by hydroxylation. Cholesterol passes through the outer mitochondrial membrane and after crossing the outer chamber reaches the inner membrane and cristae. The cleavage system includes 20- & 22-hydroxylases and 20, 22-lyase (Christensen 1975) present in the inner membrane and cristae of the mitochondrion. 20α-hydroxylation is one of the rate-limiting first events in the side-chain cleavage of cholesterol, leading to steroid synthesis (Neaves 1977 a, b); it involves one or both carbons involved in the C-20 to C-22 bond (Hall & Young 1968, Hall 1970, Guraya 1980). The terminal oxidase in hydroxylations,—Cytochrome P-450 occurs in below detectable levels mostly and in trace quantity in dog and man (Christensen 1975). The resulting $\triangle^5$ pregnenolone (figure 31, numeral 8) passes into the closely located SER or the cytoplasm surrounding the SER and enters the $\triangle^4$ (or progesterone) or $\triangle^5$ (or dehydroepiandrosterone as in human testis) steroidogenic pathway according to the species studied (figure 31, numeral 9). How exactly pregnenolone gains exit from the mitochondrion is not known and it is also not clear where the newly synthesized T finds itself in the

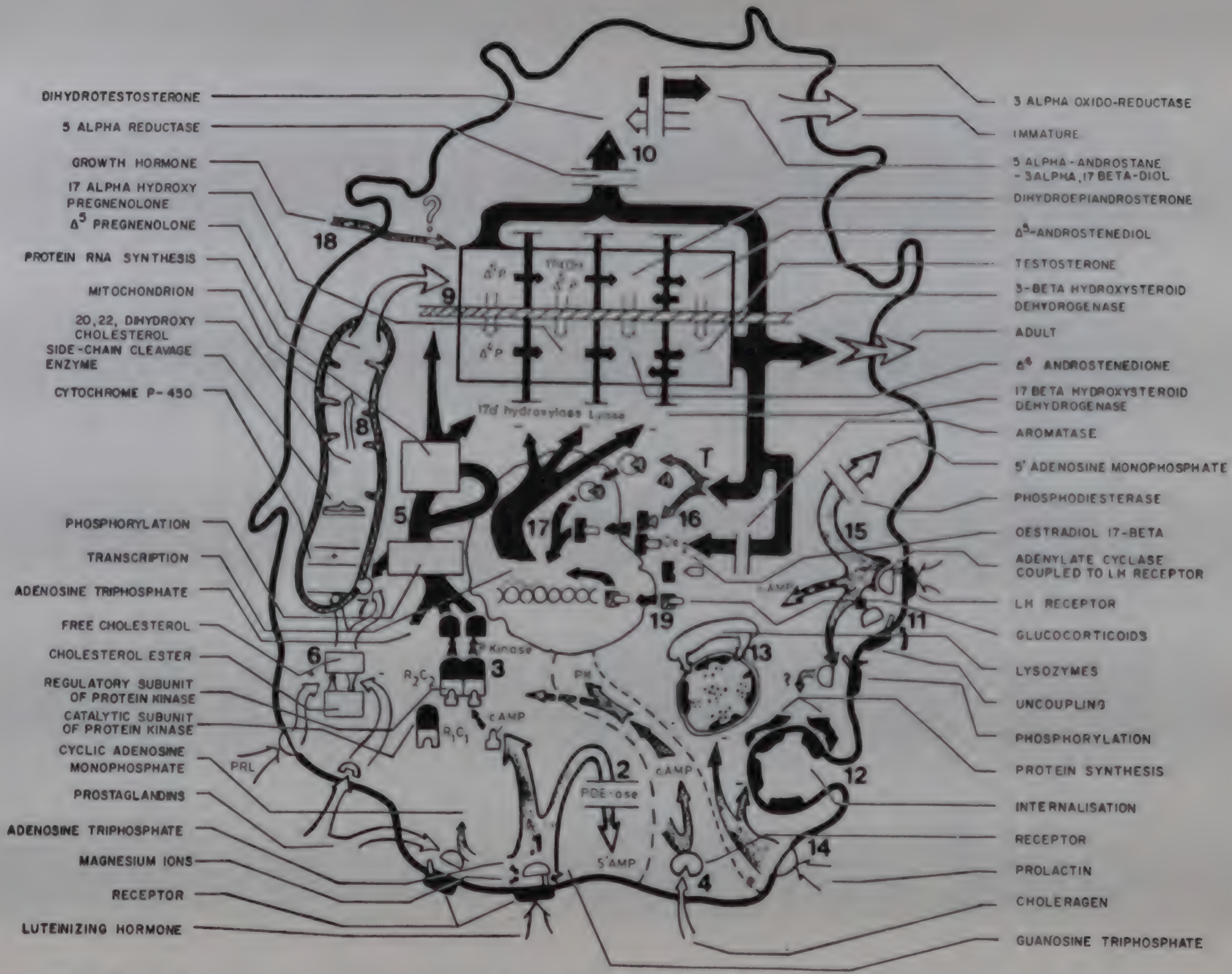


Figure 31. A diagrammatic representation of various biochemical events in a Leydig cell (after Purvis & Hansson 1981, modified)

Leydig cell. It may be in the cytoplasm outside the SER when one can expect it to diffuse out having bound itself to a carrier protein synthesized on the polysomes in the cytoplasm. If, on the other hand, it is in the membrane of the SER or within the cavity of the SER (which is tubular), no explanation is forthcoming as to how exactly the steroid hormone is secreted.

The part played by LH at the plasma membrane level of the Leydig cell is very different from that of FSH as the latter has no receptors on the Leydig cell; Means and Huckins (1974) found no specific bindings of FSH to Leydig cell membrane. The LH gonadotropin gets coupled to adenylate cyclase through GTP mediation (figure 31, numeral 1). This enzyme is the catalytic agent transforming ATP into cAMP for which magnesium ions are necessary. The cAMP intracellularly bonds itself to the regulatory subunit of protein kinase and releases the active catalytic subunit from it (figure 31, numeral 3). This latter PK subunit catalyses phosphorylation of proteins especially near steroidogenic areas in the cell leading towards RNA transcription and protein synthesis. It was pointed out that this newly-synthesized protein phosphorylated by PK may help in transporting free cholesterol released from its esters and entering the mitochondrion for side-chain cleavage.

10h *Peculiarities of Leydig Cell Population*

One of the peculiarities of Leydig cell population (in rat) is that its cells are not functionally synchronised (Purvis *et al.* 1978a); there is a certain compartmentalization of functions. They exhibit intercellular differences in 3β-HSD activity (as seen in histochemically-stained preparations). With regard to the hormone stimulation of cholera toxin (see Dufau *et al.* 1978) in the Leydig cell there appears to exist a functional compartmentalization of cAMP and PK in it and that causes differential action of hCG and choleragen on cAMP and steroidogenesis. Differences in intensity of fluorescence are also seen among cells of the same population. One part of the Leydig cell population may be 'switched off' when the other part is sensitive to LH stimulation and many of the key steroidogenic enzymes are gonadotropin dependent. The other feature in which cells are not synchronised is in the number of LH receptors per cell at its periphery; the receptor is a protein with a small phospholipid content in it (Purvis & Hansson 1981). The testicular feminised male rat is genetically insensitive to androgen as it has no androgen receptors in the target tissue; in these as a result, the circulating level of LH is elevated 8 times the normal. The Leydig cell membrane also shows a highly reduced number of receptors. This reduction in the number of LH receptors is brought about by a process of 'internalization' as described by Conn *et al.* (1978) in ovarian luteal cells. An uncoupling of the receptor (figure 31, numeral 11) from the adenylate cyclase is a possible prerequisite before internalization can take place (Purvis & Hansson 1981, p. 103); they also refer to both bound and unbound LH receptors being internalized by endocytosis (p. 123). It may be mentioned that uncoupling may not be the only method of reducing intracellular cAMP; special substances may also appear which interfere with the activity of adenylate cyclase.

10i *Secretion of Testosterone and Other Steroid Hormones from Seminal Epithelium*

Among vertebrates, the Leydig cells do not seem to be the only source of testosterone

synthesis and secretion. Lofts (see Discussion, p. 30, Hansson *et al.* 1976) described that in the soft-shelled turtle, there is a complete separation of activity between the interstitial tissue and the seminiferous tubules. If the seminiferous tubules are separated and incubated with radioactive pregnenolone and FSH added to it, there is a greater production of testosterone while addition of LH does not cause the same effect. If LH is added to the whole testis incubate, testosterone production is stimulated. It is interesting to note that the seminiferous tubules can secrete testosterone and Sertoli cells are implicated as the site of synthesis. The term steroid synthesis is usually restricted to the reaction starting with cholesterol.

It has been brought out by Setchell (1978) that the seminiferous tubules can not only convert progesterone and pregnenolone into 17α-hydroxyprogesterone, androstenedione and testosterone but also into other compounds not formed by the Leydig cells, viz., (1) 20α-hydroxypregn-4-en-3-one, (2) 5α-pregnane-3,20-dione, (3) 5α-pregnane-3β, 20 α-diol, and (4) 5α-pregnane-3β-ol-20-one.

The exact significance of this is still unclear.

10j *Hormones and the Leydig Cells*

Purvis (1978), Purvis *et al.* (1978b) and Purvis and Hansson (1981) give a comprehensive idea of the effects of FSH, LH, prolactin, testosterone, oestrogen, growth hormone and glucocorticoids on Leydig cells. Treatment of some hypophysectomised rats (probably so in other mammals too) with FSH caused maximum testicular responsiveness to LH *in vivo* (Odell & Swerdloff 1975) and maximum production of testosterone *in vitro* (Beurden *et al.* 1976) or LH receptors per testis (Chen *et al.* 1977). These results appear unconvincing as de Kretser *et al.* (1971) showed that Leydig cells have no FSH receptors and that FSH preparations available so far to workers are contaminated with LH (Purvis *et al.* 1978?, in press). In this connection, Purvis *et al.* (1978b) brought out in reply to a question of Arslan that in inframammalian species FSH is the main hormone causing LH stimulation, Purvis replied that some of the FSH effects may be due to LH contamination (p. 263). The Norwegian workers have brought out that a pretreatment of FSH with LH antiserum abolished the capacity of FSH to stimulate the secretion of androgen and, therefore, the effect of FSH on the Leydig cells according to them is mainly due to LH contamination and not to an intrinsic LH-like activity. For maintaining the steroidogenic machinery in rats, it was found that both prolactin and LH were necessary; they acted synergically and prolactin did not seem to act by itself. Leydig cells show prolactin receptors and prolactin increases LH receptors in them. Like LH, prolactin also shows a biphasic effect on Leydig cells; in small doses, it is stimulatory and in large ones, it is inhibitory. Oestrogen has a direct negative effect on Leydig cells. Both oestrogen and androgen receptor are found on the Leydig cells. A negative effect noted above is also seen in a nonaromatizable DHT propionate and very likely both these negative effects are not one and the same. Leydig cells also secrete significant quantities of oestradiol into circulation (Lipsett 1976), and how much of this is a contribution from the Sertoli cell is unclear and under certain conditions, it may be that oestrogen has an inhibitory effect on the Leydig cells. No studies have been undertaken to see if there are growth hormone receptors on Leydig cells. Glucocorticoids have a large number of receptors on Leydig cells.

Growth hormone has a stimulatory (figure 31, numeral 18) and glucocorticoid an inhibitory (numeral 19) effect of LH on Leydig cells.

The finding that Leydig cell has a specific oestradiol receptor (van der Molen *et al.* 1973) may have some bearing on Hall's (1970) suggestion that the seminiferous epithelium produces an oestrogen which may function in the feedback control of steroidogenesis.

11 THE MALE DUCT SYSTEM IN A MONKEY

The duct system in a male mammal or monkey in particular shows four parts: the rete testis, the vasa efferentia, the epididymis and the vas deferens; of these, the rete testis is testicular and the other three parts are extra-testicular. The epididymis has attracted considerable attention as one which may help in the control of male fertility without impairing libido and potentia.

11a *The Epididymis*

The epididymis is usually studied under three heads according to classical description, viz., the caput, the corpus and the cauda. As the caput traverses the entire length of the testis lobe in the guinea pig, this nomenclature defeated its own purpose and Glover and Nicander (1971) therefore called the parts, the initial, the middle and terminal segments. They have also shown how the three segments differ histologically.

The initial segment and the testis seem to be interdependent; injection of cadmium caused injury to blood vessels supplying the proximal part of the initial lobe (Gunn *et al.* 1963). The blood vessels of the initial lobe become more permeable on cadmium injection (Waites & Setchell 1966). If the blood circulation of the initial lobe is interfered with, the testes show damage and the vasa efferentia get blocked (Harrison & MacMillan 1954).

Functionally, the first two segments appear to help in sperm maturation while the last segment is for the storage of spermatozoa. Prakash *et al.* (1979) have given an account of light microscope studies of the epididymis of the rhesus monkey. This description does not differ substantially from that of Glover and Nicander (1971).

According to Prakash *et al.* (1979), the seminiferous tubules open into the rete testis by means of tubuli recti which are merely extensions of rete. It is not clear from the description whether both ends of the tubule open into the rete through the tubuli or one end of it may end blindly in some. They also confirm the observation of Roosen Runge (1961), that at the region the tubule enters the tubulus, there is a knob of Sertoli cells playing a definite role (see p. 13).

The rete testis lying within the tunica albuginea at the region of the vasa efferentia open into the latter. The epithelium of the tubulus rectus shows columnar cells; the epithelium of the rete in the mediastinum and the tunica shows mostly squamous cells and a few cuboid cells also.

The vasa efferentia [3–7 in number in the rhesus monkey according to Setchell (1978), 12–17 in the same species according to Prakash *et al.* (1979)] show two types of cells; predominant are the nonciliated type, showing a brush border. The ciliated cell is the pale cell type (vide: Epididymis) with many mitochondria; kinocilia with basal granules are noticed apically of these cells.

11b *The Histology of the Epididymis*

The initial segment shows tall cells with long straight stereocilia; spermatozoa, when present, are few in number. EM studies disclose the presence of micropinocytotic vesicles at the surface and multivesicular bodies. The middle segment has bent stereocilia and the lumen is wider and spermatozoa are present. Supranuclear vacuoles are seen. Ultrastructural studies show the presence of 'macropinocytotic' vesicles and large multivesicular bodies. The epithelium in the terminal segment is shorter than in the other two segments and the stereocilia are low and thin. EM studies show micropinocytotic vesicles and probably there is not much absorption in this region. Spermatozoa abound in this region and show a spiral arrangement (Prakash *et al.* 1979).

With regard to cell types, there are three distinct types: (a) the principal cell (majority in number), (b) the pale cell and (c) the basal cell. The principal cell is of the tall columnar variety and negative images of Golgi are seen in the supranuclear region. In the pale cell, the apical part is broad and the basal part slender; mostly these cells are found in the middle segment and least so in the terminal one. These are devoid of stereocilia and are probably secretory. They seem to help in the acidification of the fluid in this part of the epididymis (Levine & Marsh 1971). The basal cell is close to the basement membrane and never reaches th lumen of the epididymis. These cells exhibit micropinocytotic activity. Pobably they also contain lysosomes and function histolytically.

The terminal segment continues into the vas deferens. This shows according to the above authors, three types of cells: Type I, Type II and basal cells. Type II cells resemble the pale cells of the epididymis. Probably the vas deferens is also secretory in addition to conveying the spermatozoa. In man, the maturation of spermatozoa in fact takes place in the vas deferens according to Bedford (1975) but a later account by Hinrichsen and Blaquier (1980) states that it is in the epididymis as in other mammals.

11c *The Mammalian Epididymal Physiology*

This part of the male duct system plays a vital physiological role bringing about maturation of spermatozoa and Prasad (1973) has summarised the findings; as a result, they show migration of the cytoplasmic droplet, change in membrane characteristics, increase in specific gravity, increase in fertilizing capacity and changes in pattern of movement or motility.

The plasma of the epididymis in which the sperm are found shows the following biochemical constituents: (a) electrolytes, (b) enzymes, (c) lipids, (d) glyceryl-phosphoryl-choline (GPC), (e) carnitin, (f) sialic acid, (g) proteins, and (h) steroid hormones. It is not clear how these play on the floating spermatozoa in their dynamic biochemical role (Prasad & Rajalakshmi 1976).

Sialo mucoproteins have been found in the epididymis of all the species so far examined. Sialic acid bonding with spermatozoa was highest in the initial segment and gradually decreased while bonding with epididymal plasma was greatest in the terminal segment. This inverse relationship may have a role in the development and maintenance of the fertilizing ability of spermatozoa. Further, the authors state

that the sialoproteins may stabilize the acrosome, the plasma and the acrosomal membranes during maturation (Gupta *et al.* 1974). An antiandrogen reduces the secretory activity of the epididymis, the lowering of sialic acid resulting in the concomitant loss of the integrity of the acrosome. Sialomucoproteins also maintain ionic balance of the epididymal plasma. Crabo (1965) brought out that Na^+ and K^+ are absorbed in the proximal segment while K^+, chloride and Na^+ are absorbed in the terminal one. This loss is made good by the sialic acid.

With regard to GPCA and carnitin, Prasad and Rajalakshmi (1976) feel that the latter may not give a clue to maturation of spermatozoa.

They point out that the epididymis exhibits different androgen threshold and this duct part gets its testosterone from three sources: from the general body circulation, from the rete fluid and it has a built-in capacity to synthesize *in vitro* (Hamilton 1971, 1972, 1975); and why it needs such a large concentration of androgen, is unclear.

Setty (1979) has reviewed the usefulness of antiandrogen in the control of male fertility and advocates a method to induce reversible 'functional' sterility. This means that spermatogenesis must go on (without impairing libido and potentia) but the extragonadal sperm must be rendered sterile (Singh *et al.* 1970). Prasad and Porter (1976) reported recently that it was premature to decide one way or the other. It is known that in the epididymis, the spermatozoa acquire ability to fertilize (maturation) and continue to be stored in the terminal segment. It is also known that the milieu in the epididymis can be interfered with chemicals (de Kretser 1974, Setty & Kar 1976). The growth, differentiation and functional maturation of the epididymis are androgen dependent (Riar *et al.* 1977). Workers from time to time have reported that in the epididymis, there are histological differences in the three parts, the secretory and absorptive function also vary, there are also other biochemical differences and peculiarly, variation of blood circulation in different parts is seen. Castration affects adversely the epididymal morphology, cytology and histochemistry and these are set right by androgen therapy (Jones & Glover 1973, 1975). It was brought out that the epididymis can get its androgen from three sources but a fourth source could be from the venous-arterial transfer in the pampiniform plexus (Einer-Jensen 1974, Einer-Jensen & Waites 1977).

The 5α-dehydrotestosterone appears to mediate the action of testosterone. Receptors in the cytoplasm and the nucleus of the epididymal epithelial cells help in the process as reported by Hansson *et al.* (1975), Danzo and Eller (1975, 1976), and Blaquier (1974) in different animals. It was also brought out that RNA and protein synthesis was androgen sensitive (Blaquier 1975) and whether castration causes the disappearance of the recepters as reported by Calandra *et al.* (1975) needs confirmation (see Calandra *et al.* 1977).

It was found that the sperm gain capacity to fertilize in the distal part of the epididymis; in the rabbit, such mature spermatozoa are found in the distal region of the middle segment; in the rat and hamster, such sperm are found in the proximal region of the terminal segment and in man, maturation also takes place in the epididymis according to Hinrichsen and Blaquier (1980) and not always in vas deferens. In view of the above findings, Orgebin Crist *et al.* (1975) observed that the capacity to mature is intrinsic to spermatozoa and as such the environment does not influence them and it is age that determines it. Salisbury (1962) brought out that androgen

inhibits or delays spermatozoal maturation till they reach the distal region or that the same is caused by differences in ionic composition in the initial segment. Obviously the above statements do not endow the epididymis with a positive role in sperm maturation.

Orgebin Crist and others have re-examined this problem. Rabbit rete testis spermatozoa when incubated with the terminal segment fluid failed to fertilize; terminal lobe spermatozoa did not lose the fertilizing ability when treated with rete testis fluid. If the epididymis is maintained in organ culture, the spermatozoa from the distal part of the middle segment did not lose fertilizing ability for 1 hr or 3–4 days when androgens were added to the culture. Spermatozoa from the initial segment under those experimental conditions failed to fertilize (Orgebin Crist *et al.* 1976b). The same set of authors also brought out that the steroidal antiandrogen cyproterone acetate when added in *in vitro* culture of epididymal tubules, the tubule spermatozoa lost their fertilizing ability.

Using GPC, sialic acid and carnitine as parameters, it was thought that the high level of GPC would indicate the secretory activity of the epididymal epithelium.

With regard to sialic acid, probably it helps in the transport of spermatozoa by a lubricating action (Riar *et al.* 1973) or maintain osmotic balance in the terminal lobe or the sialoproteins may be involved in the stabilization of the acrosomal membrane (Rajalakshmi *et al.* 1976). Carnitine is not synthesized in the epididymis but is extracted from the blood. Orgebin Crist *et al.* (1976a) report that the rete testis spermatozoa exposed *in vitro* to this chemical did not make them fertile.

Voglmayr (1976) examined the adrogen control of the metabolic activity of spermatozoa with reference to the nature of binding of sperm to steroid, the metabolism of steroid by sperm and how the spermatozoan metabolism was affected by the steroid. A number of others have also examined that aspect and Hammerstedt and Amann (1976) came to the conclusion that no specific steroid could be linked to sperm function.

12 ANTIANDROGENS OR ANDROGEN ANTAGONISTS

According to Mainwaring (1977), a good antiandrogen must exhibit three properties: (i) low toxicity, (ii) high biological activity at low concentration, and (iii) it should not exhibit any hormonal activity in assays for androgens, oestrogens, progestagen or glucocorticoids. Such an ideal androgen antagonist has yet to be synthesized.

Two groups of antiandrogens are known: one group has only antiandrogenic properties [called 'pure' antiandrogens, Cyproterone (C), flutamide] and the other group is both antiandrogenic and antigonadotropic and the latter activity may be ascribed to their progestational properties (Cyproterone acetate (CA), chloromadinone acetate). The antigonadotropic activity of CA is exerted fully only in females; in the male, part of antigonadotropic activity is neutralised by the antiandrogenic effect (Neumann *et al.* 1977).

12a *Cyproterone Acetate*

Antiandrogens were first suggested as extragonadal antifertility agents by Cavazos

and Melampy (1956) and later, Whalen and Luttge (1969) suggested CA as a male contraceptive agent. CA is a steroid hormone, the strongest antiandrogen known and its structural formula is written in at least three different forms:

(i) 6-chloro-17-acetoxy-1α-2α-methylene-4,6-pregnadiene-3,20-dione

(ii) 1,2 α-methylene-6-chloro-$\triangle^{4,6}$-pregnadien-17α-ol-3,20-dione 17α-acetate

(iii) 6-chloro-$\triangle^{6}$-1,2 α-methylene-17 α-hydroxyprogesterone acetate.

This androgen antagonist is excellent for long term treatment of men as it is both antiandrogenic and antigonadotropic. Further on account of its progestational side effect, appearing as temporary gynecomastia after prolonged use, it has central inhibitory properties and therefore, it can profitably be used on men. CA appears to act by competitive inhibition of testosterone action on target sites (Neumann & von Berswordt-Wallrabe 1966) or as Steinbeck and Neumann (1971) put it, CA inhibits androgen effects mainly at the receptor sites. It can also be used as a hormonal contraceptive for women along with oestrogen.

CA suppresses spermatogenesis in all animal species in a dose dependent manner (Neumann 1977). Suppression of spermatogenesis at the stage of spermatids, or at the secondary spermatocyte or spermatid stage or something similar to hypophysectomy can be caused by starting with a low dose of CA and then increasing it. While in some animals CA causes sterility without loss of libido (rat and guinea pig), in others it inhibits libido (rabbit and man). The sterility caused by CA is the result of suppression of spermatogenesis. Working with CA, Prasad *et al.* (1970) implanted subcutaneously silastic capsule(s) liberating 232 μg/day of the drug into each male rat and they became sterile after 4 months. All the same, the weights of testes and accessory glands did not change as also the secretory function of accessory glands (fructose and citric acid); sialic acid, however, showed a definite decrease. Spermatozoa taken from initial and terminal segments of the epididymis were nonmotile and matings proved sterile and the epididymis histologically appeared degenerate (reduction in the height of the epithelium, depletion of secretory granules, cellular vacuolation and nuclear pycnosis). Prasad *et al.* (1970) postulated that since neither libido [libido in rats cannot be inhibited by CA according to Whalen and Edwards (1969)] nor accessory gland structure was affected by the drug, the epididymis could serve as a good parameter for measuring the effects of CA as it is the one that is affected. In the epididymis, sperm maturation whose one facet is the acquisition of motility is suppressed (Prasad *et al.* 1970). The terminal segmont (cauda) of the epididymis where sperm maturation is noticed greatly has a higher threshold of androgens than the initial segment (caput) for its physiology. The CA caused a depletion of sialic acid in the initial and terminal segments, their histology was impaired, the poor staining of acrosome of the epididymal spermatozoa with PAS followed by loss of motility, viability and fertilizing ability of epididymal spermatozoa (Rajalakshmi *et al.* 1971). Obviously the epididymis appears to be most sensitive to CA. If however, the capsules are removed, a gradual restitution of fertility occurs after 30 days. Prasad *et al.* (1973) feel that the differential androgen threshold' hypothesis described above may lead to the induction of functional sterility in the human male. If this occurs, CA could be used as a contraceptive agent in the male. All the same, Steinbeck and Neumann (1971) say that they have strong reservation against the use of CA as a contraceptive agent for men.

12b *α-Chlorohydrin* (*3-chlor*-1,2 *propanediol*; *U*5897)

Another drug that has been used is α-chlorohydrin to control fertility. Waites (1976) stated that many workers showed that α-chlorohydrin in low doses interfered specifically with cauda spermatozoa. Setty *et al.* (1970) in testing this drug on male rhesus monkeys for antifertility measures used orally a total dose of 3800 mg spread over a period of time. They found that it was not antispermatogenic and did not impair the endocrine integrity of the testis; even toxic doses did not affect the testis in rhesus monkeys (see Discussion, p. 111, Glover 1976). Braz *et al.* (1976) have administered *sc* a total dose of 3900 mg of the drug spread over a period of 30 days to male langur monkeys (*Presbytis entellus*?) this affected the seminal epithelial and epididymal histology; there was disruption of spermatogenesis. Effect on spermatozoa, however, was not studied. While a large body of workers is of the opinion that the action of α-chlorohydrin is post-testicular, probably confined to the epididymis, there is on earlier record by Samojlik and Chang (1970) in addition to that of Braz *et al.* (1976) describing a total inhibition of spermatogenesis; majority of Leydig cells remained unchanged.

Hoffer *et al.* (1972) administered a single high dose of 140 mg/kg of α-chlorohydrin orally to rats and found that the drug interfred with fluid absorption in the initial segment (caput) of the epididymis.

The toxic effect of α-chlorohydrin is well known and therefore an analogue of it, trimethyl-phosphate was tried. This was alse not recommended by Orgebin Crist as a useful contraceptive agent (see General Discussion, p. 194 in *J. Reprod. Fertil.* Suppl. **24** 1976); she suggested that in order to control fertility in the male one should interfere with sperm viability in the cauda or with maturation in the more proximal part of the epididymis.

After taking into account the results of workers on CA, Neumann *et al.* (1970) came to the conclusion that fertility could only be controlled if spermatogenesis is impaired or only when suboptimal levels of androgen are found in the epididymis and therefore no 'functional' sterility could be brought about. One should read the arguments brought out by Prasad *et al.* (1971/72) in favour of CA as a suitable male contraceptive as against the arguments of Neumann *et al.* (1970) stated above.

In reviewing the mechanism of antiandrogen actions, Tymoczko and Liao (1976) noted that it :

(i) may affect RNA, DNA and protein synthesis,
(ii) may prevent androgen and DHT formation or their receptor bindings,
(iii) may alter receptors so that their binding is impaired, or produce androgen and antiandrogen receptor complexes which may upset nuclear events.

After taking all these facts into consideration, Setty (1979) summed up by saying that the extragonadal antifertility action of antiandrogens will require:

(i) lowering of synthesis of androgen in the testis or production of ABP or
(ii) lowering of local androgen synthesis due to fall in circulating androgen level or
(iii) preventing dihydrotestosterone formation and androgen binding to receptors.

The Norwegian School (Purvis *et al.* 1978c) has brought ut that both antiandrogns C and CA have direct effect on the testis. Immature male rats treated with CA showed a dose dependent decrease in testis and epididymal weights. Epididymal contents of ABP similarly decreascd (FSH action on Sertoli cells). Plasma testosterone was also inhibited (LH action on Leydig cells). Plasma corticosterone level was also suppressed. These changes occurred when the plasma levels of FSH and LH (pituitary action) remained unchanged. Since relatively low doses of antiandrogens inhibited androgen secretion, it may not be a receptor mediated phenomenon. *In vitro* metabolism studies brought out that the antiandrogen acted at the enzyme (3β-HSD) level. The effects of the antiandrogens may be due to "a complex balance of androgen receptor, glucocorticoid, progestational and direct enzyme effects unrelated to any of these properties" and which one of these plays a major role depends on the dosage, duration and age of the animals (Purvis *et al.* 1978c).

In this connexion, it may be relevant to remember the findings of Neumann *et al.* (1970) with regard to the effects of small and large doses of CA. Administration of a low dose having both antiandrogenic and antigonadotropic effects, the feed back mechanism is not disturbed as the two effects appear to balance each other. With higher doses, the antigonadotropic activity supervenes as seen in the fall of gonadotropic and androgen secretion and the volume of testis also goes down (Neumann *et al.* 1970).

13 THE SPERMATOZOON

Bloom and Fawcett (1976) consider the spermatozoon as a holocrine secretory product of the seminiferous tubule. Historically there is a reference to the semen containing seed in the Old Testament (Genesis). It was in the year 1677 that a young Dutch medical student Johan Hamm saw spermatozoa in semen and A. van Leewenhoek reported about it in 1679. Two Schools of opinion developed after this: the 'ovists' believed that the sperm nourished the famale seed to develop; the 'animists' saw a miniature embryo in the sperm. In the human sperm head, it was called the 'homunculus'. After the discovery of the egg by van Baer in 1827, the penetration of the sperm into the cgg was reported by M. Barry.

In a sectional view of the testis, the light microscope reveals that the head of the spermatozoon is imbedded in the Sertoli cell cytoplasm; in EM studies, it is found that the integrity of the Sertoli cell plasma membrane is maintained and the head is actually present in the Sertoli cell recesses in the luminal side.

The sperm morphology was described by early cytologists as visible under the light microscope; then followed EM studies but they did not use highly sophisticated methods of fixation which considerably obfuscated their results and when these became available, the intricate morphology of the spermatozoon was studied. Fawcett (1958) gave an account of his EM studies in 1958 and recently, Phillips (1975) has given an updated account. Their findings can be briefly summarised as follows:

The spermatozoon exhibits three parts : (1) the head with acrosomal cap which may assume a variety of shapes, (2) the middle piece having a sheath of mitochondria

which provide the motive force for the movement of the gametes and the parts 1 and 2 are connected by a short neck or connecting piece, and (3) the tail composed of fibres and articulating with the head by a 'ball and socket' joint. Phillips (1975) described a head and tail in the sperm. While the acrosome and nucleus constitute the head region, the rest of the sperm described above comes under the tail region.

The sperm head shows a variety of shapes depending on the animal species studied. The human sperm head is oval (figure 32a) seen on the flat and appears pyriform when seen on the edge (figure 32b). It is usually described as having a nucleus (figure 32b), an acrosome, a head and postnuclear cap. In addition, the sperm of some rodents possess a refractile process, — the rod or perfortorium. It was correctly shown by Clermont and Leblond (1955) that the nucleus occupies all the part of the head and that the acrosome is inconspicuous in a mature sperm. A vacuole(s) may be present in the head of the human sperm; so also in that of bull and stallion. Fawcett (1958) feels that it may not be an essential organelle of the sperm. As the spermatid gradually matures (Fawcett & Phillips 1969), the diffuse chromatin condenses and the nucleus undergoes change in shape and the factors causing these are not known. Probably the microtubules surrounding the spermatid nucleus (the so-called manchette) may play a part in the above (Fawcett & Phillips 1969).

13a *The Acrosome*

The term acrosome was probably introduced by Lenhossek (1898) to describe a spherical granule within the idiosome during the differentiation of the sperm. The investigations of Clermont and Leblond (1955) brought out that the acrosome had tinctorially an outer and inner zone; the head cap is formed by the outer zone while the acrosome is formed out of the more deeply staining inner zone. Later EM studies have confirmed these findings. According to Fawcett (1958), no evidence of enzyme activity was noticed in the acrosome. According to Phillips (1975) the acrosome is built by the Golgi apparatus; it appears first as proacrosomal granules between the Golgi and the nucleus. The granules unite to form a single granule. This granule changes its shape according to the species studied. In the rabbit, monkey, bat and man, it closely follows the contour of the nucleus. This cup-shaped secretion granule contains hydrolytic enzymes which latter help in the penetration of the sperm into the egg through the corona and the zona. The plasma membrane covering the acrosome may show tubules and vesicles. EM studies have disclosed that the acrosomal content is not as homogeneous as was supposed to be. In the hanuman langur monkey, my EM studies have disclosed that the acrosome forms a cap as it were in the anterior half of the sperm nucleus in the advanced spermatid. Bedford (1967) brought out that the subacrosomal space was absent in the human sperm; Zamboni *et al.* (1971) who examined a few monkeys and man, reported that a subacrosomal space was present in man. In my EM studies of the langur male monkey, I have noticed the presence of a subacrosomal space in the spermatid thereby resembling more the human spermatid.

A perfortorium, referred to previously, is absent from the sperm of man and monkey (Zamboni *et al.* 1971). I have also not noticed such a structure in the anterior end of the langur monkey sperm.

A postnuclear cap described by early workers in the human spermatozoan is

absent not only in the human but in a number of genera investigated by Clermont and Leblond (1955).

The region just posterior to the sperm head is called neck (figure 32c). The centriole is present in this region. The classical cytologists described that the centriole divides into two and one retains the typical centriole nature and the other is modified into a basal body of the flagellum and of its attachment to sperm head. Fawcett's EM studies (1958) have not differed from the above observation; the cross-banded body in the neck represents the distal centriole modified into a basal body. Phillips (1975) brought out that the posterior region of the nucleus in the neck is either flat or concave. Then follows a thin electron lucid zone which is followed by a basal plate and which is continuous with 9 fibre bundles of the neck or middle piece; the middle piece bundles are merely a continuation of the above. According to Fawcett ad Phillips (1969), the nine columns form in relation to the distal centriole or basal body and this appears later. Some mammalian sperm exhibit only the proximal centriole (Fawcett & Phillips 1969). In the rat, Woolley and Fawcett (1973) have brought out that both centrioles disappear.

The middle piece is the part between the basal body and the annulus (the so-called ring annulus). It consists of axial bundle of fibrils (axial filament) and the mitochondrial helix. The axial bundle (figure 32d) is now known to consist of 11 fibrils in all, two single fibrils in the centre and nine double fibrils around them. In a trans-sectional view of the rat middle piece, one notices that inside the mitochondrial sheath, there are two sets of nine fibrils encircling the central two. The outer set

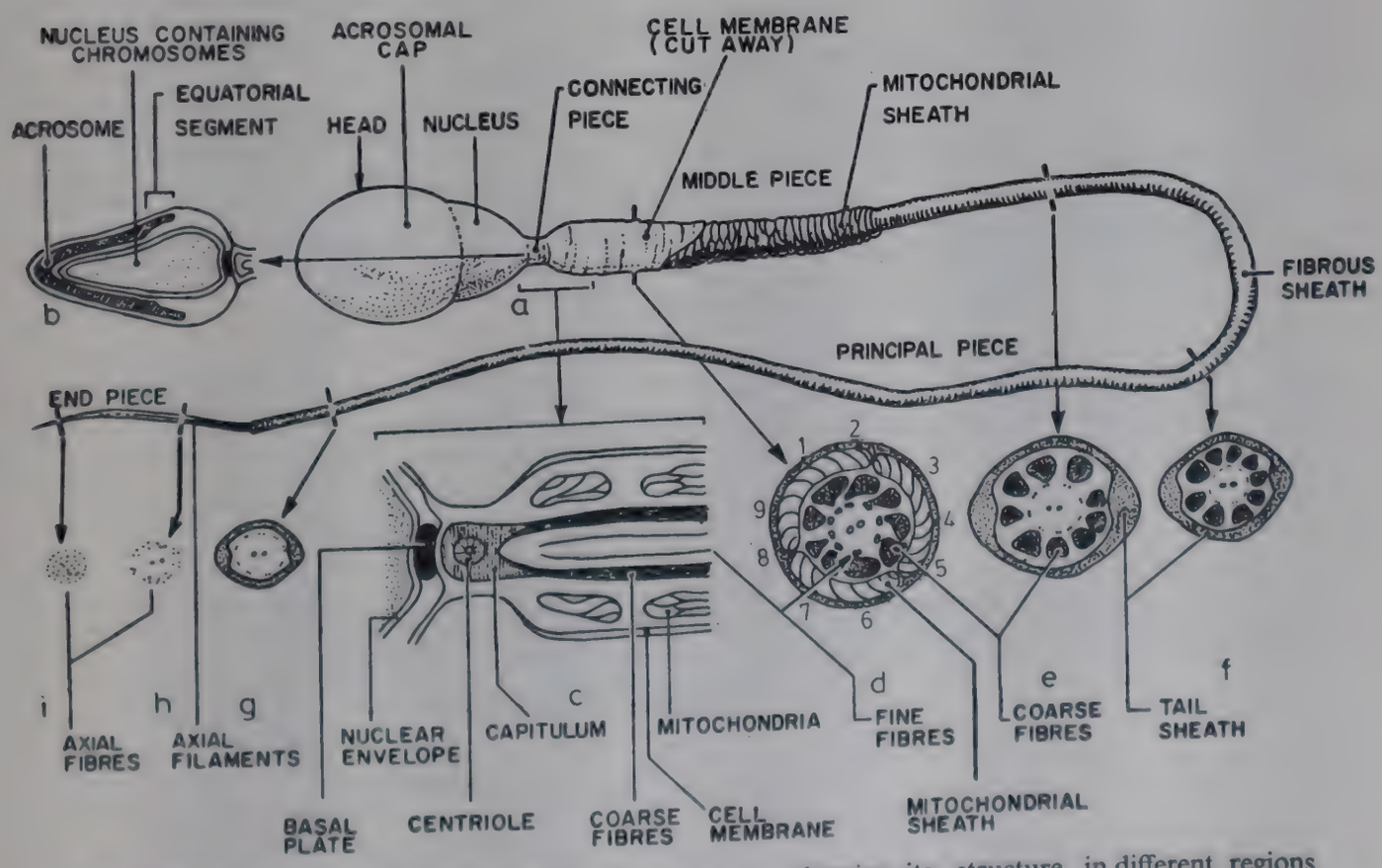


Figure 32. Morphology of the human spermatozoan showing its structure in different regions (after Hancock 1971, In Human Reproduction, Paladin)

is larger in size than the inner; also the outer are coarse fibres while the smaller set has fine fibres. Moreover, of the outer, three are larger than the rest (figure 32d); of these three, one is on one side and the other two on the opposite side. If this single large one is numbered 1 (figure 32d), then the other two larger ones are 5 and 6 counting the other large ones clockwise; a line passing from 1 and between 5 and 6 would divide the middle piece into two bilateral halves.

The mitochondria (figures 32c, 32d) have been attributed with energy production and it is not known how chemical energy is translated into motility.

The annulus is situated at the junction of the middle piece and the principal piece or tail. This has been called ring centriole as it was thought to arise from the centriole. According to Fawcett (1958) the annulus is not a division product of the centriole as the latter is not noticed to divide. It is also not clear if the annulus is present in the mature human sperm and Anberg (1957) does not represent it in his figures.

The transverse sectional views (figures 32 e–i) give an idea of the internal structure of the remaining part of the tail.

13b *The Residual Body*

I have reproduced figures (see 33A–F) from Fawcett *et al.* (1971) showing the differentiation of the spermatozoon sloughing the cytoplasmic portion as the residual body. These figures bring out the formation of the acrosome and the condensation

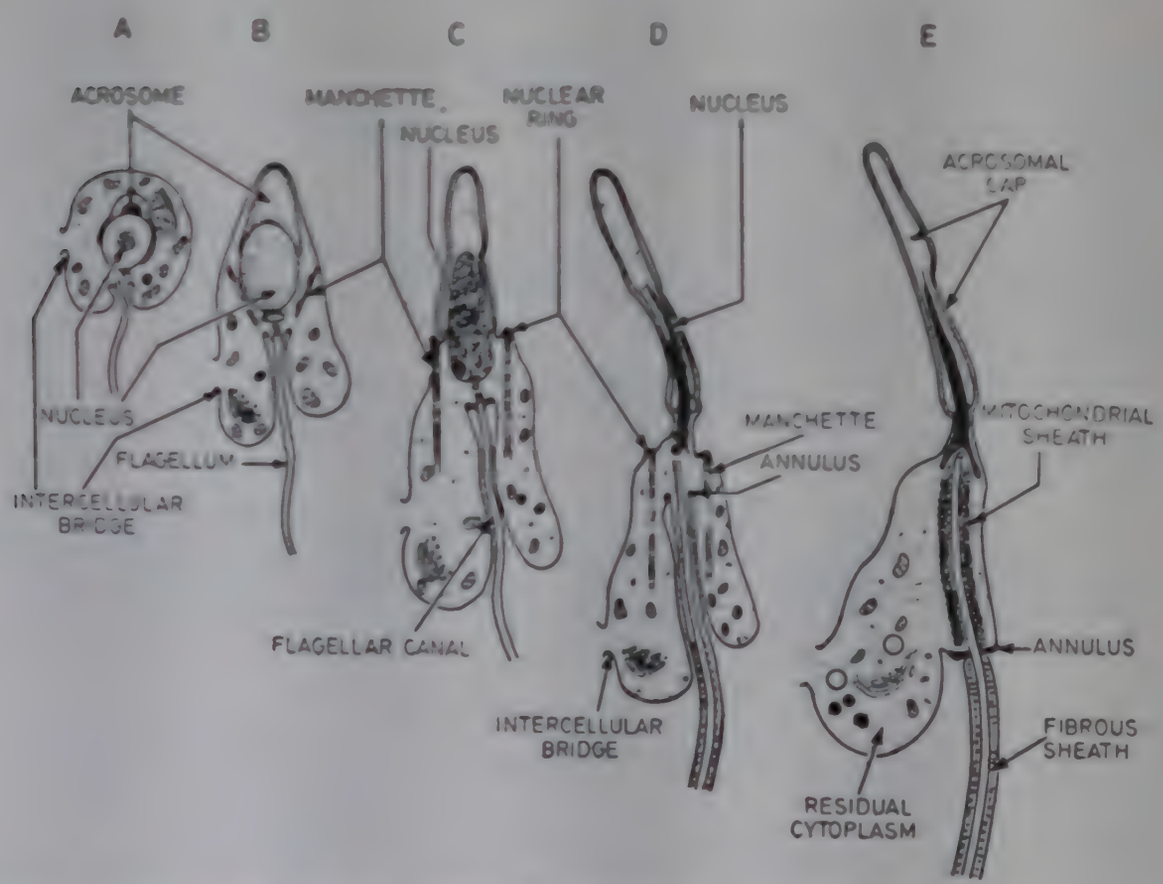


Figure 33. Fate of the cytoplasm during the passage of spermatid into spermatozoon, (after Fawcett *et al.* 1971, modified)

of the nucleus capped by the acrosome. The appearance of the manchette and its subsequent disappearance are also noticed in figures B and D. The elongation of the cell is seen (B–E) and during this process, most of the cytoplasm is thrown to one side (E) and is connected subsequently with the spermatozoon by a stalk. When the spermatozoon finally disengages itself from the Sertoli cell, it leaves its cytoplasm in the latter (as the residual body) as the connecting stalk breaks and a 'cytoplasmic droplet' remains usually along the intermediate piece and consists of endoplasmic reticulum, Golgi apparatus and hydrolytic enzymes (Dott & Dingle 1968) in the droplet. These features of the spermatid differentiation are clearly shown diagrammatically by Fawcett (1972).

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KEY TO LETTERING

A	ype A spermatogonium
A′	androgen
A_0	undividing reserve stem cell
A_1–A_4	renewing stem cells
a	activator
AAC	additional artery to caput epididymis
ABP	androgen binding protein
ACE	artery to caput epididymis
ACR	acrosome
ADC	(adlum. compt.) adluminal compartment
AP	anterior pituitary
ATP	adenosine triphosphate
B	type B spermatogonium
BL	basal lamina
BV	blood vessel
CAE	caput epididymis
cAMP	cyclic AMP
CAT	one of the caudal hair-pin bends of a tubule before opening into rete testis
CEP	lymph channel in caput epididymis
CM	cell membrane
CNS	central nervous system
COE	corpus epididymis
CR	cistern of reticulum
CPA	centripetal artery
CRT	one of the cranial hair-pin bends of a tubule before opening into rete testis
CSC	C-shaped sex-cord
CTE	connecting vessel between internal spermatic artery and caudal epididymal artery
CUE	cauda epididymis
DE	efferent ducts
DHEA	dehydroepiandrosterone
DHT	dihydrotestosterone
DI	diakinesis
DR	efferent ducts from rete
EA	epididymal artery
EP	epididymis
EPD	epididymal duct
ETR	extra-testicular rete
FBA	artery to the fat body
FCM	fusion of cell membranes
FIL	filaments
FSH	follicle stimulating hormone
GC	Golgi complex
GCE	germ cell
GH	growth hormone
i	inhibitor
ICA	deep parenchymal artery
ICS	intercellular space
IEA	inferior epididymal artery
IMT	immature tubule
In	intermediate spermatogonium

ISA	internal spermatic artery
ITR	intra-testicular rete
JC	junctional complex
JCH	each half of the separated junctional complex re-positions itself by the side of zygotene spermatocyte
L	leptotene spermatocyte
LC	Leydig cell
LCT	loose connective tissue
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LIP	lipid
LNU	lobulated nucleus
LTA	lymph vessel in tunica albuginea
LV	lymphatic vessel
LYMSI	lymphatic sinusoid
LYMSP	lymph space
m	mitosis
MED	mediastinum
MIT	mitochondria
MOD	modulation in the tubule
MRB	manchette in the residual body
mRNA	messenger RNA
MTS	microtubules
MYL	myoid layer
NLE	nucleolus
NU	nucleus
OE_2	oestradiol 17β
P	pachytene spermatocyte
PDS	poorly developed septulae
PEN	parietal epithelium
PIG	pigment
PL	preleptotene or leptotene spermatocyte
PP	pampiniform plexus
PPI	posterior pituitary
PSTR	projection of Sertoli cells into tubulus rectus
PV	peripheral vein
R	cytoplasmic receptor
RS	resting spermatocyte
RB	residual body
RBC	cytoplasm of residual body
RBL	'rete blastema'
RC	protein kinase
RER	rough endoplasmic reticulum
RI	ribosomes
RNA	ribose nucleic acid
RT	rete testis
R + C	regulator and catalytic subunits of RC
SA	spermatic artery
SC	scrotal wall
SCY	syncytium
SEA	superior epididymal artery
SEC	Sertoli cell cytoplasm
SEP	septula from the tunica to mediastinum
SER	smooth endoplasmic reticulum

SK	satellite karyosome
SN	Sertoli cell nucleus
SOR	site of reversal of 'wave' in the tubule
SPN	nucleus of spermatozoon
SS	secondary spermatocytes
SSJ	Sertoli-Sertoli cell tight junction
ST	seminiferous tubule
T	testosterone
TA	tunica albuginea
TC	testicular capsule
TE	testis
TL	testis lobule
TP	parenchyma of testis
TR	tubulus rectus
TV	tunica vasculosa
TVP	tunica vaginalis parietal
TVV	tunica vaginalis visceral
VA	vasal artery
VD	vas deferens
VE	vasa efferentia
VIE	visceral endothelium
VV	vasal vein
Z	zygotene spermatocyte

int. acell. layer, ext. acell. layer } internal and external acellular layers

Fig. 10 I–VI: cell associations in the wall of the seminiferous tubule
Figs. 11 & 13: I–XIV stages of the spermatogenic cycle: 1–19 in fig 11: steps in spermiogenesis
Fig. 19: arrows indicate entry of tracer
Fig. 12: 1–8 represent steps in spermiogenesis

ERRATA LIST

Page	*Para*	*Read*	*Instead of*
Contents	3	Anatomy of the mammalian testis	Anatomy of the mammalian
3	1	(Hundeiker 1972)	(Hundeiker 1972
	2	centrifugal	cetrifugal
6	1	(Arey 1965)	(Arcy 1965)
9	Fig. 6 legend	to enter	two enter
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45	Fig. 23 legend	hormone receptor	homone receptor
52	4	INHIBIN	INHIBNI
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Professor L S Ramaswami is a distinguished and versatile zoologist of the country. He received his D.Sc. degree from the University of Madras and taught at the Universities of Mysore and Rajasthan. His early researches include the embryology of bats and the cranial osteology of fishes, amphibia and reptilia, notably structural peculiarities of the skull and the Weberian ossicles of the family Cyprinidae in relation to phylogeny, induction of spawning in catfish using pituitary hormones. He started a fish physiology laboratory at Bangalore in 1954, using the air-breathing catfish ***Heteropneustes fossilis*** as the experimental system and also extended his interests to the comparative endocrinology of the frogs, particularly the skipper frog, ***Rana cyanophlyctis.***

He was responsible for building a modern endocrinology laboratory with an electron microscope and a well-equipped animal house at the University of Rajasthan, Jaipur. His group produced excellent work on the reproductive biology of the langur monkey and the pusillanimous ***Loris.***

Professor Ramaswami's works drew the scientific attention of stalwarts abroad, and he subsequently established close research collaboration with many of them.

Professor Ramaswami has published over 100 technical papers and his works are extensively cited in reviews and books. He was invited to several important international symposia in India and abroad to present technical papers.

He was elected a Fellow of the Indian National Science Academy in 1957 and was honoured with the coveted Sunder Lal Hora Medal (1975), in recognition of his outstanding contributions. He presided over the Zoology Section of the Indian Science Congress at Cuttack in 1962. He delivered the first "Dr (Mrs) Shantha Rao Memorial Oration" lecture at the Institute of Research for Reproduction, Bombay, 1980. At their Third Annual Conference of the Society for Reproductive Biology and Comparative Endocrinology held at the Sri Venkateswara University, Tirupati, June 1983, the members honoured Professor Ramaswami with a souvenir; he being the doyen of the subjects.

For Professor Ramaswami age is only an event for the calendar. Embers of his scientific enquiry keep glowing unquenched.